Fatty Acids during Pregnancy and Cardio-metabolic Outcomes in Mothers and their Children

The Generation R Study

Aleksandra Jelena Vidakovic

Fatty Acids during Pregnancy and Cardio-metabolic Outcomes in Mothers and their Children

The Generation R Study

Aleksandra Jelena Vidakovic

Acknowledgements
The general design of the Generation R Study is made possible by financial support from the Erasmus Medical Center, Rotterdam, the Erasmus University Rotterdam, the Netherlands. Organization for Health Research and Development (ZonMw), the Nether-lands Organisation for Scientific Research (NWO), the Ministry of Health, Welfare and Sport and the Ministry of Youth and Families. Research leading to the results described in this thesis has received funding from the European Union's Seventh Framework Pro-gramme (FP7/2007-2013), project EarlyNutrition under grant agreement n°289346.

The work presented in this thesis was conducted in the Generation R Study Group, in close collaboration with the Departments of Epidemiology, Pediatrics, Obstetrics and Gynaecology, Erasmus Medical Center, Rotterdam, the Netherlands.

Publication of this thesis was kindly supported by the Department of Epidemiology, the Generation R Study Group and the Erasmus University Rotterdam. Financial support by the Dutch Heart Foundation for the publication of this thesis is gratefully acknowledged.

ISBN: 978-94-6299-455-3
Cover illustrations: D. Grabez & N. Ilic
Thesis layout: Ridderprint BV
Thesis printing and cover layout: Ridderprint BV

Fatty Acids during Pregnancy and Cardio-metabolic Outcomes in Mothers and their Children

The Generation R Study

Vetzuren tijdens de zwangerschap en cardio-metabole uitkomsten bij moeders en hun kinderen

Het Generation R Onderzoek

Thesis

to obtain the degree of Doctor from the
Erasmus University Rotterdam
by command of the
Rector Magnificus

Prof.dr. H.A.P. Pols

and in accordance with the decision of the Doctorate Board

The public defense shall be held on
Tuesday 15th of November 2016 at 9:30 hours

by

Aleksandra Jelena Vidakovic

born in Zvornik, Serbia

Erasmus University Rotterdam

Doctoral committee

Promotors:	Prof.dr. V.W.V. Jaddoe
	Prof.dr. E.A.P. Steegers
Other members:	Prof.dr. I.K.M. Reiss
	Prof.dr. E.F.C. van Rossum
	Prof.dr. H.A. Smit
Copromotor:	Dr. R. Gaillard

Paranymphs:	S. Santos
	E. Voerman

Contents

Manuscripts based on this thesis

Chapter 2.1

Vidakovic AJ, Jaddoe VW, Gishti O, Felix JF, Williams MA, Hofman A, Demmelmair H, Koletzko B, Tiemeier H, Gaillard R. Body mass index, gestational weight gain and fatty acid concentrations during pregnancy: the Generation R Study. Eur J Epidemiol. *2015;30(11):1175-85.*

Chapter 2.2

Vidakovic AJ, Jaddoe VW, Steegers EA, Gaillard R. Maternal fatty acid profiles, hemodynamic adaptations and gestational hypertensive disorders. The Generation R Study. *Submitted*

Chapter 3.1

Vidakovic AJ, S Santos, Williams MA, Duijts L, Hofman A, Demmelmair H, Koletzko B, Jaddoe VW, Gaillard R. Maternal plasma n-3 and n-6 polyunsaturated fatty acid concentrations during pregnancy and subcutaneous fat mass in infancy. *Obesity (Silver Spring). 2016;24(8):1759-66.*

Chapter 3.2

Vidakovic AJ, Gishti O, Voortman T, Felix JF, Williams MA, Hofman A, Demmelmair H, Koletzko B, Tiemeier H, Jaddoe VW, Gaillard R. Maternal plasma PUFA concentrations during pregnancy and childhood adiposity: the Generation R Study. *Am J Clin Nutr. 2016;103(4):1017-25.*

Chapter 3.3

Vidakovic AJ, Gishti O, Steenweg-de Graaff J, Williams MA, Duijts L, Felix JF, Hofman A, Tiemeier H, Jaddoe VW, Gaillard R. Higher maternal plasma n-3 PUFA and lower n-6 PUFA concentrations in pregnancy are associated with lower childhood systolic blood pressure. *J Nutr. 2015;145(10):2362-8.*

Chapter 3.4

Vidakovic AJ, Jaddoe VW, Demmelmair H, Koletzko B, Gaillard R. Maternal plasma polyunsaturated fatty acid levels during pregnancy and childhood lipids and insulin levels. *Nutr Metab Cardiovasc Dis. In press*

Chapter 1

General introduction

General introduction

Introduction

Cardiovascular disease is the leading cause of death globally.[1] Despite major improvement in cardiovascular health, coronary heart disease remains responsible for 17.3 million deaths per year.[1] The developmental origins hypothesis suggests that cardiovascular diseases have their origins in prenatal life and in early childhood.[2] Adverse exposures, acting at different stages of fetal and early postnatal development, lead to permanent adaptations in the structure, physiology and function of various organ systems. This early programming contributes to short-term survival, but increases the susceptibility of cardio-metabolic diseases later in life. Maternal nutritional status is a major factor influencing fetal environment.[3]

Maternal nutrition during pregnancy might have significant consequences on later health and well-being. The Dutch Hunger Winter study, which results were first published in 1976 demonstrated the effects of intrauterine deprivation on subsequent adult health.[4] The Dutch famine study, which studied the influence of severe undernutrition during pregnancy on offspring outcomes, showed for the first time that undernutrition during gestation increases the risk of many diseases, including obesity, heart disease, and diabetes. This study has provided crucial support and fundamental insights for the growing field of the developmental origins of health and disease. However, nowadays the Western countries are facing a burden of malnutrition and a large amount of epidemiological studies indicated that a deficient provision of nutrients during pregnancy results in long-lasting effects on the health of mothers and their children. Thus, this suggests that improved maternal nutrition with essential nutrients during gestation may reduce the risk of chronic diseases in adults. However, not much is known about the influence of specific maternal nutrients on offspring outcomes.

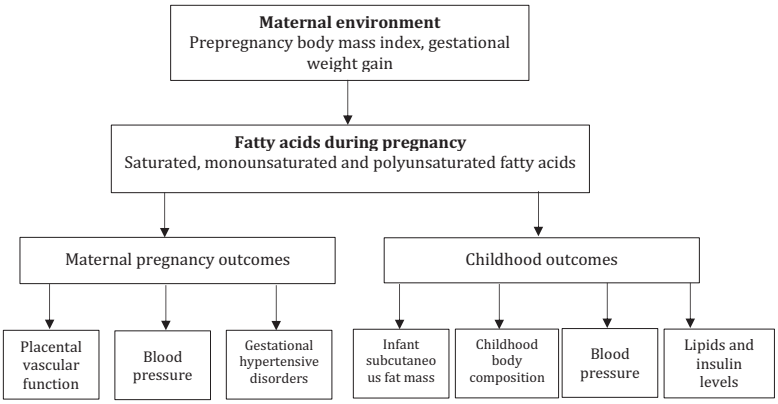
Maternal fatty acids during pregnancy are essential nutrients for the developing fetus. Fatty acids are molecules composed of a hydrocarbon chain with a carboxylic acid at one end. They can be classified by how many double bonds are present in the hydrocarbon tail. The fatty acids without carbon-carbon double bonds are classified as saturated fatty acids (SFAs), while those containing carbon-carbon double bonds are classified as unsaturated, such as monounsaturated (MUFA) and polyunsaturated fatty acids (PUFAs).[5] Two types of PUFAs are of particular interest, because they are used in the body, but they cannot be synthesized de novo by humans. These two types of essential fatty acids are n-3 and n-6 PUFAs.[6] Consequently these fatty acids must be derived from the diet. The majority sources of SFAs and MUFAs come mainly from animal and vegetable fats and oils. N-3 PUFAs are obtained from two dietary sources,

seafood and plant oils, while the main source of n-6 PUFAs are animal fat. PUFAs and their derivatives have important roles in cell membrane synthesis and regulation of gene expression. They are also precursors of hormones and eicosanoids such as prostaglandins, prostacyclin's, thromboxanes and leukotrienes. These signalling molecules are important for the regulation of several cellular processes, including inflammation, immunity, coagulation and vasoconstriction.[7] In adult populations, clinical and epidemiological studies have shown that higher SFAs and MUFAs are strong predictors of hypertension and coronary heart disease mortality.[8,9] On the contrary, n-3 PUFAs, especially α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), and linoleic acid (LA), a n-6 PUFA, protect against cardiovascular disease.[10]

During pregnancy, the fetus depends on maternal fatty acids availability.[11] Fatty acids can cross the placental syncytiotrophoblast as free fatty acids derived from triglycerides and phospholipids. This process involves several fatty acid transport and binding proteins.[12] Placental transfer of PUFAs is an important factor in determining fetal growth and development.[13] Studies have demonstrated that an adverse maternal fatty acids profile, with higher levels of SFA and MUFA, but lower levels of PUFA, are associated with increased risk of gestational hypertensive disorders.[14,15] Moreover, evidence from randomized control trials has demonstrated that n-3 PUFA supplementation during pregnancy results in higher birthweight and a decreased risk of preterm delivery.[16] However, thus far not much is known about the influence of maternal fatty acids status during pregnancy on long-term offspring consequences.

In summary, maternal malnutrition during pregnancy may adversely affect long term cardio-metabolic health of offspring. In less extreme environments, maternal fatty acids status is an important factor associated with fetal development and birth outcomes. Identifying the associations of maternal fatty acids status during pregnancy with childhood cardio-metabolic outcomes, may help to develop future preventive strategies that improve cardio-metabolic health in future generations by optimizing maternal nutritional status during pregnancy. Therefore, studies presented in this thesis were designed to identify the associations of maternal fatty acids status during pregnancy with cardio-metabolic health outcomes of the mothers and their children. The overall hypothesis of this thesis is given in the **Figure 1.1**.

Figure 1.1. Overview of hypothesis for the associations of maternal fatty acid levels and cardio-metabolic health of mothers and their children



Maternal environment

Maternal prepregnancy obesity is a major public health problem worldwide, and is associated with adverse maternal and fetal pregnancy outcomes.[17] Prepregnancy obesity, defined as a body mass index (BMI) ≥30 kg/m^2, is a risk factor for obstetric complications, such as gestational diabetes, preeclampsia and adverse birth outcomes.[18,19] Excessive gestational weight gain is also related to adverse pregnancy and offspring outcomes.[20] During pregnancy, both higher prepregnancy BMI and excessive gestational weight gain could result in a higher risk of decreased insulin sensitivity and therefore decreased ability of insulin to supress lipolysis, which results in increase free fatty acid concentrations.[21] Previous studies have reported that a higher BMI during pregnancy is associated with higher levels of SFA and lower levels of n-3 PUFA.[22,23] It has also been suggested that obese women appear to have poorer dietary quality compared with non-obese women, which may result in an inadequate fatty acids profile.[24] However, no previous study has examined the associations of maternal prepregnancy body mass index and gestational weight gain during pregnancy with different maternal fatty acids levels.

Vascular adaptations during pregnancy

Gestational hypertensive disorders are the most common medical problem during pregnancy, complicating approximately 7% of all pregnancies. They are a major

cause of maternal, fetal and neonatal morbidity and mortality.[25,26] Animal studies demonstrated that higher intake of SFAs during pregnancy may cause placental vascular damage and affect the regulation of placental blood flow, leading to pregnancy-related maternal hemodynamic maladaptation and gestational hypertensive disorders.[27] The role of fatty acids on gestational hypertensive disorders in human pregnancy is poorly studied. During pregnancy, it has been suggested that higher maternal levels of SFA and MUFA, but lower levels of PUFA, especially n-3 PUFAs, are associated with higher maternal blood pressure.[14,15] Thus far, results of studies focused on the associations of maternal fatty acids and gestational hypertensive disorders seem to be inconsistent and most studies have assessed only total free fatty acids, while individual fatty acids have not been well studied.[28] Also, not much is known about direct effect of fatty acids on maternal blood pressure and placental function throughout pregnancy. Thus, examining the associations between different maternal fatty acid levels with hemodynamic adaptations during pregnancy and the occurrence of gestational hypertensive disorders may provide further insight in the pathways underlying these associations.

Childhood outcomes

PUFAs and offspring body composition

Childhood obesity is a major public health problem.[29] During pregnancy, an adequate maternal intake of nutrients contributes to early adaptations in fetal development.[30] PUFAs are an essential component for normal growth and early PUFAs exposure might influence adipose tissue development through activation of peroxisome proliferator-activated receptors, which promote adipogenesis.[31] Previous animal and human studies suggested that n-3 PUFA promotes weight loss; although the results of studies are inconsistent.[32-34] Studies in children also suggested that exposure to n-3 PUFAs during pregnancy is associated with a lower risk of childhood obesity, whereas maternal n-6 PUFAs during pregnancy increases adiposity in childhood.[35,36] Thus far, most studies used childhood body mass index as a measure of adiposity. Not much is known about the association between maternal plasma PUFA levels with specific body fat measures from early infancy onwards. From a preventive perspective, it is important to obtain a better understanding of the associations of maternal plasma n-3 and n-6 PUFA levels with detailed offspring fat mass measures from early infancy onwards.

PUFAs and offspring cardio-metabolic outcomes

Maternal nutrition during pregnancy may have long-term adverse consequences for offspring cardio-metabolic health.[37] Several studies during pregnancy have shown that higher maternal PUFA levels are associated with higher blood pressure, total cholesterol and insulin levels in children.[38-40] However, the evidence for a possible programming effect of PUFAs during pregnancy on later cardio-metabolic risk in childhood is still sparse and inconclusive. Thus, understanding the relationship between the maternal PUFA status during pregnancy and childhood systolic and diastolic blood pressure as well as total-cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, insulin and c-peptide levels in the offspring, could identify specific maternal PUFA requirements during pregnancy which affect cardio-metabolic outcomes in the offspring.

General aim of this thesis

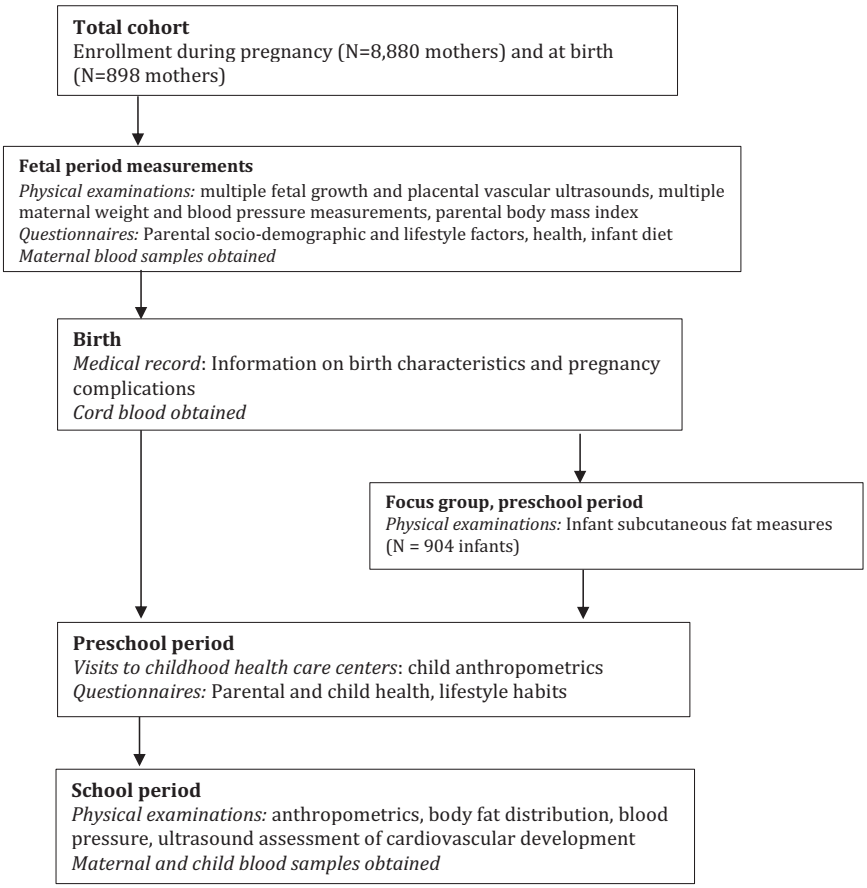
The general aim of this thesis was to identify the associations of maternal plasma fatty acid levels during pregnancy with maternal hemodynamic adaptations during pregnancy and cardio-metabolic outcomes in childhood.

General design

The studies presented in this thesis were embedded in the Generation R Study, a population based prospective cohort study from fetal life until young adulthood in Rotterdam, The Netherlands.[41] The Generation R Study is designed to identify early environmental and genetic determinants of growth, development and health in fetal life and childhood. All pregnant women living in the study area with a delivery date between April 2002 and January 2006 were eligible for enrolment in this study. Enrolment was aimed at early pregnancy, but was possible until the birth of the child. In total, 9,778 mothers were enrolled in the study, of whom 8880 (91%) were included during pregnancy (**Figure 1.2**). Assessments were planned in early pregnancy (<18 weeks of gestation), mid-pregnancy (18 - 25 weeks of gestation) and late pregnancy (≥25 weeks of gestation), and included parental physical examinations, maternal blood and urine collection, fetal ultrasound examinations, and self-administered questionnaires. In the preschool period, from birth to 4 years of age, data collection was performed in all children by questionnaires and visits to the routine child health care centers. All children were invited to a dedicated research center in the Erasmus

MC-Sophia Children's Hospital to participate in detailed body composition and cardiovascular follow-up measurements at the age of 6 years. Measurements during this visit included anthropometrics, body composition, cardiovascular development and blood sample collection.

Figure 1.2. Design and data collection in the Generation R Study



Outline of this thesis

The objectives are addressed in several studies presented in this thesis. In **Chapter 2**, studies on maternal fatty acids status during pregnancy on gestational adaptations are described. In **Chapter 2.1**, we examined the associations of prepregnancy body

mass index and gestational weight gain with plasma fatty acid concentrations in mid-pregnancy. The associations of maternal plasma fatty acid concentrations with longitudinal blood pressure development and placental vascular function throughout pregnancy and the risks of gestational hypertensive disorders are described in **Chapter 2.2.**

In **Chapter 3**, we present studies focused on the associations of maternal n-3 and n-6 PUFA levels with infant and childhood cardio-metabolic outcomes. The associations of maternal plasma n-3 and n-6 PUFA concentrations during pregnancy with infant subcutaneous fat mass measures are presented in **Chapter 3.1.** In **Chapter 3.2** and **3.3**, we examined whether maternal plasma n-3 and n-6 PUFA concentrations during pregnancy are associated with childhood adiposity and childhood blood pressure, respectively. **Chapter 3.5** describes the association of maternal plasma n-3 and n-6 PUFA levels during pregnancy with lipid and insulin levels in childhood.

Finally, **Chapter 4** provides a general discussion in which the studies described in this thesis are described in broader context, and implications and suggestions for future research are discussed.

References

1. Mendis S, Puska P and Norrving B. Global atlas on cardiovascular disease prevention and control. World Health Organization. 2011.
2. Gluckman PD, Hanson MA, Cooper C and Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med. 2008;359:61-73.
3. Wu G, Bazer FW, Cudd TA, Meininger CJ and Spencer TE. Maternal nutrition and fetal development. J Nutr. 2004;134:2169-72.
4. Roseboom T, de Rooij S and Painter R. The Dutch famine and its long-term consequences for adult health. Early Hum Dev. 2006;82:485-91.
5. FD Gunstone FN. Lipids in foods: chemistry, biochemistry and technology. 2013
6. Wassall SR and Stillwell W. Polyunsaturated fatty acid-cholesterol interactions: domain formation in membranes. Biochim Biophys Acta. 2009;1788:24-32.
7. Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. Science. 2001;294:1871-5.
8. Xu J, Eilat-Adar S, Loria C, Goldbourt U, Howard BV, Fabsitz RR, Zephier EM, Mattil C and Lee ET. Dietary fat intake and risk of coronary heart disease: the Strong Heart Study. Am J Clin Nutr. 2006;84:894-902.
9. Wang L, Manson JE, Forman JP, Gaziano JM, Buring JE and Sesso HD. Dietary fatty acids and the risk of hypertension in middle-aged and older women. Hypertension. 2010;56:598-604.
10. Valensi P. Hypertension, single sugars and fatty acids. J Hum Hypertens. 2005;19 Suppl 3:S5-9.
11. Hornstra G, Al MD, van Houwelingen AC and Foreman-van Drongelen MM. Essential fatty acids in pregnancy and early human development. EurJ Obstet Gynecol Reprod Biol. 1995;61:57-62.

12. Larque E, Demmelmair H, Gil-Sanchez A, Prieto-Sanchez MT, Blanco JE, Pagan A, Faber FL, Zamora S, Parrilla JJ and Koletzko B. Placental transfer of fatty acids and fetal implications. Am J Clin Nutr. 2011;94:1908S-1913S.
13. Hornstra G. Essential fatty acids in mothers and their neonates. Am J Clin Nutr. 2000;71:1262S-9S.
14. Oken E, Ning Y, Rifas-Shiman SL, Rich-Edwards JW, Olsen SF and Gillman MW. Diet during pregnancy and risk of preeclampsia or gestational hypertension. Ann Epidemiol. 2007;17:663-8.
15. Dayana Farias, Fernanda Rebelo, Ilana Eshriqui, Jaqueline Lepsch, Thatiana Pinto, Ana Franco-Sena, and NL, Kac G and Maternal Serum Fatty Acids are Positively Associated with Systolic Blood Pressure Changes during Pregnancy: Longitudinal Study. The FASEB Journal. 2015;29.
16. Imhoff-Kunsch B, Briggs V, Goldenberg T and Ramakrishnan U. Effect of n-3 long-chain polyunsaturated fatty acid intake during pregnancy on maternal, infant, and child health outcomes: a systematic review. Paediatr Perinat Epidemiol. 2012;26 Suppl 1:91-107.
17. Wolfe H. High prepregnancy body-mass index--a maternal-fetal risk factor. N Engl J Med. 1998;338:191-2.
18. Chu SY, Callaghan WM, Kim SY, Schmid CH, Lau J, England LJ and Dietz PM. Maternal obesity and risk of gestational diabetes mellitus. Diabetes Care. 2007;30:2070-6.
19. Cedergren MI. Maternal morbid obesity and the risk of adverse pregnancy outcome. Obstet Gynecol. 2004;103:219-24.
20. Carreno CA, Clifton RG, Hauth JC, Myatt L, Roberts JM, Spong CY, Varner MW, Thorp JM, Jr., Mercer BM, Peaceman AM, Ramin SM, Carpenter MW, Sciscione A, Tolosa JE, Saade GR, Sorokin Y, Eunice Kennedy Shriver National Institute of Child H and Human Development Maternal-Fetal Medicine Units N. Excessive early gestational weight gain and risk of gestational diabetes mellitus in nulliparous women. Obstet Gynecol. 2012;119:1227-33.
21. Catalano PM. Obesity, insulin resistance, and pregnancy outcome. Reproduction. 2010;140:365-71.
22. Micallef M, Munro I, Phang M and Garg M. Plasma n-3 Polyunsaturated Fatty Acids are negatively associated with obesity. Br J Nutr. 2009;102:1370-4.
23. Karlsson M, Marild S, Brandberg J, Lonn L, Friberg P and Strandvik B. Serum phospholipid fatty acids, adipose tissue, and metabolic markers in obese adolescents. Obesity (Silver Spring). 2006;14:1931-9.
24. Jarvandi S, Gougeon R, Bader A and Dasgupta K. Differences in food intake among obese and non-obese women and men with type 2 diabetes. J Am Coll Nutr. 2011;30:225-32.
25. Nijdam ME, Janssen KJ, Moons KG, Grobbee DE, van der Post JA, Bots ML and Franx A. Prediction model for hypertension in pregnancy in nulliparous women using information obtained at the first antenatal visit. J Hypertens. 2010;28:119-26.
26. Steegers EA, von Dadelszen P, Duvekot JJ and Pijnenborg R. Pre-eclampsia. Lancet. 2010;376:631-44.
27. Liang C, DeCourcy K and Prater MR. High-saturated-fat diet induces gestational diabetes and placental vasculopathy in C57BL/6 mice. Metabolism. 2010;59:943-50.
28. Hubel CA, McLaughlin MK, Evans RW, Hauth BA, Sims CJ and Roberts JM. Fasting serum triglycerides, free fatty acids, and malondialdehyde are increased in preeclampsia, are positively correlated, and decrease within 48 hours post partum. Am J Obstet Gynecol. 1996;174:975-82.
29. Han JC, Lawlor DA and Kimm SY. Childhood obesity. Lancet. 2010;375:1737-48.
30. Barker DJ, Osmond C, Kajantie E and Eriksson JG. Growth and chronic disease: findings in the Helsinki Birth Cohort. Ann Hum Biol. 2009;36:445-58.

31. Massiera F, Saint-Marc P, Seydoux J, Murata T, Kobayashi T, Narumiya S, Guesnet P, Amri EZ, Negrel R and Ailhaud G. Arachidonic acid and prostacyclin signaling promote adipose tissue development: a human health concern? J Lipid Res. 2003;44:271-9.
32. Huang XF, Xin X, McLennan P and Storlien L. Role of fat amount and type in ameliorating diet-induced obesity: insights at the level of hypothalamic arcuate nucleus leptin receptor, neuropeptide Y and pro-opiomelanocortin mRNA expression. Diabetes Obes Metab. 2004;6:35-44.
33. Madsen L, Petersen RK and Kristiansen K. Regulation of adipocyte differentiation and function by polyunsaturated fatty acids. Biochim Biophys Acta. 2005;1740:266-86.
34. Awad AB, Bernardis LL and Fink CS. Failure to demonstrate an effect of dietary fatty acid composition on body weight, body composition and parameters of lipid metabolism in mature rats. J Nutr. 1990;120:1277-82.
35. Donahue SM, Rifas-Shiman SL, Gold DR, Jouni ZE, Gillman MW and Oken E. Prenatal fatty acid status and child adiposity at age 3 y: results from a US pregnancy cohort. Am J Clin Nutr. 2011;93:780-8.
36. Moon RJ, Harvey NC, Robinson SM, Ntani G, Davies JH, Inskip HM, Godfrey KM, Dennison EM, Calder PC, Cooper C and Group SWSS. Maternal plasma polyunsaturated fatty acid status in late pregnancy is associated with offspring body composition in childhood. J Clin Endocrinol Metab. 2013;98:299-307.
37. Langley-Evans SC. Nutrition in early life and the programming of adult disease: a review. J Hum Nutr Diet. 2015;28 Suppl 1:1-14.
38. Jochems SH, Gielen M, Rump P, Hornstra G and Zeegers MP. Potential programming of selected cardiometabolic risk factors at childhood by maternal polyunsaturated fatty acid availability in the MEFAB cohort. Prostaglandins Leukot Essent Fatty Acids. 2015;100:21-7.
39. Rump P, Popp-Snijders C, Heine RJ and Hornstra G. Components of the insulin resistance syndrome in seven-year-old children: relations with birth weight and the polyunsaturated fatty acid content of umbilical cord plasma phospholipids. Diabetologia. 2002;45:349-55.
40. Voortman T, van den Hooven EH, Braun KV, van den Broek M, Bramer WM, Chowdhurry R and Franco OH. Effects of polyunsaturated fatty acid intake and status during pregnancy, lactation, and early childhood on cardiometabolic health: A systematic review. Prog Lipid Res. 2015;59:67-87.
41. Jaddoe VW, van Duijn CM, Franco OH, van der Heijden AJ, van Iizendoorn MH, de Jongste JC, van der Lugt A, Mackenbach JP, Moll HA, Raat H, Rivadeneira F, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC and Hofman A. The Generation R Study: design and cohort update 2012. Eur J Epidemiol. 2012;27:739-56.

Chapter 2

Maternal factors

Chapter 2.1

Prepregnancy body mass index, gestational weight gain and fatty acids in pregnancy

Aleksandra Jelena Vidakovic, Vincent W.V. Jaddoe, Olta Gishti, Janine F. Felix, Michelle A. Williams, Albert Hofman, Hans Demmelmair, Berthold Koletzko, Henning Tiemeier, Romy Gaillard

Adapted from Eur J Epidemiol. 2015;30(11):1175-85

Abstract

Obesity during pregnancy may be correlated with an adverse nutritional status affecting pregnancy and offspring outcomes. We examined the associations of prepregnancy body mass index and gestational weight gain with plasma fatty acid concentrations in mid-pregnancy. This study was embedded in a population-based prospective cohort study among 5,636 women. We obtained prepregnancy body mass index and maximum weight gain during pregnancy by questionnaires. We measured concentrations of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), n-3 polyunsaturated fatty acid (n-3 PUFA) and n-6 polyunsaturated fatty acid (n-6 PUFA) at a median gestational age of 20.5 (95% range: 17.1–24.9) weeks. We used multivariate linear regression models. As compared to normal weight women, obese women had higher total SFA concentrations [difference: 0.10 standard deviation (SD) (95% Confidence Interval (CI): 0, 0.19)] and lower total n-3 PUFA concentrations [difference: - 0.11 SD (95% CI: - 0.20, - 0.02)]. As compared to women with sufficient gestational weight gain, those with excessive gestational weight gain had higher SFA concentrations [difference: 0.16 SD (95% CI: 0.08, 0.25)], MUFA concentrations [difference: 0.16 SD (95% CI: 0.08, 0.24)] and n-6 PUFA concentrations [difference: 0.12 SD (95% CI: 0.04, 0.21)]. These results were not materially affected by adjustment for maternal characteristics. Our results suggest that obesity and excessive weight gain during pregnancy are associated with an adverse fatty acids profile. Further studies are needed to assess causality and direction of the observed associations.

Introduction

Overweight and obesity are major public health problems across all ages and populations in Western countries.[1-8] Previous studies reported a prevalence of up to 30% among pregnant women.[9,10] Obesity during pregnancy is associated with increased risks of adverse pregnancy outcomes, including gestational hypertensive disorders, gestational diabetes, fetal death and large size for gestational age infants.[11-14] Recent studies suggest that maternal obesity and excessive weight gain during pregnancy are also associated with adverse cardiovascular outcomes in the offspring.[14,15] The mechanisms underlying these associations may involve fetal cardiovascular and metabolic adaptations in response to increased transport of specific fatty acids to the placenta and fetus.[16] Increased body mass index is associated with dyslipidemia and insulin resistance during pregnancy, which may lead to increased circulating free fatty acids concentrations.[17] Among adolescents and adults, it has been shown that a higher body mass index is associated with higher concentrations of saturated fatty acids (SFAs), lower concentrations of n-3 polyunsaturated fatty acids (PUFAs) and higher n-6/n-3 PUFA ratio.[18,19] A previous study in the United States among 129 pregnant women suggested that women who were obese before pregnancy had lower concentrations of docosahexaenoic acid, an n-3 PUFA, and arachidonic acid, an n-6 PUFA, whereas concentrations of eicosapentaenoic acid, an n-3 PUFA, did not differ by prepregnancy body mass index categories.[20] This study did not have information on other fatty acid concentrations available.

Therefore, we examined in a population-based prospective cohort study among 5,636 women, the associations of prepregnancy body mass index and gestational weight gain with plasma fatty acid concentrations in mid-pregnancy.

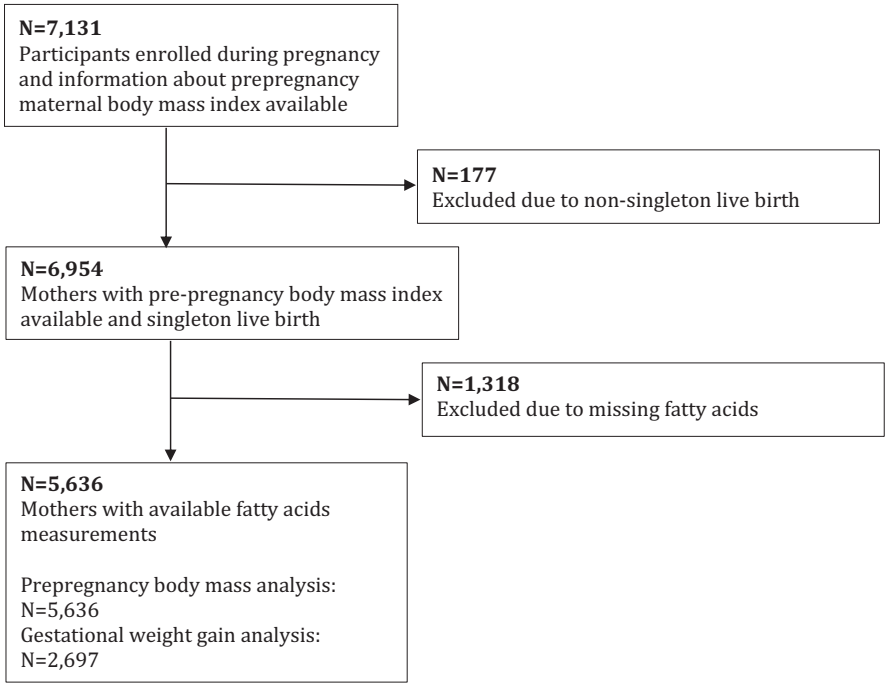
Methods

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from foetal life to adulthood in Rotterdam, the Netherlands.[21,22] The study has been approved by the Medical Ethical Committee of the Erasmus MC in Rotterdam (MEC 198.782/2001/31). All mothers gave written consent. Pregnant women with an expected delivery date from April 2002 to January 2006 were enrolled in the study. In total, 7,131 mothers were enrolled during pregnancy, of whom 6,954 had information on prepregnancy body mass index measurements available

and gave birth to singleton live born children. Mid-pregnancy fatty acids concentrations were measured among 5,636 women (Flow chart is given **Figure 2.1.1**).

Figure 2.1.1. Flow chart of the participants



Prepregnancy body mass index and gestational weight gain

At enrollment, we measured height (cm) and weight (kg) without shoes and heavy clothing, and calculated body mass index (kg/m^2). Information about weight just before pregnancy was obtained by questionnaire. In our population for analysis, 52.3% of all women were enrolled before a gestational age of 14 weeks. Correlation of prepregnancy weight, obtained by questionnaire, and weight measured at enrollment was 0.95 (*P* value<0.001). Prepregnancy body mass index was categorized into 4 categories (underweight [<20.0 kg/m^2], normal weight [20.0 –24.9 kg/m^2], overweight [25.0–29.9 kg/m^2], and obese [≥30.0 kg/m^2]). Information about maximum weight during pregnancy was available in a subset of 2,697 women and was assessed by questionnaire 2 months after delivery. According to the Institute of Medicine guidelines, we defined insufficient and excessive gestational weight gain in

relation to prepregnancy body mass index (for underweight and normal weight: total weight gain <11.5 and >16.0 kg; for overweight: total weight gain <7 and >11.5 kg; for obesity: total weight gain <5 and >9.0 kg, respectively).[23]

Fatty acid status

Venous samples were drawn at a median gestational age of 20.5 weeks (95% range: 17.1-24.9). To analyze fatty acids concentrations, EDTA plasma samples were selected and transported to the Division of Metabolic Diseases and Nutritional Medicine, Dr. von Hauner Children's Hospital, University of Munich Medical Center. After being thawed, the analysis of plasma glycerophospholipid fatty acids was performed by a sensitive and precise high throughput method. This method is suitable for applications in large epidemiological studies.[24] Based on findings from previous studies, we selected fatty acids for our analyses, which have been associated with the risk of cardiovascular and metabolic outcomes in adults, and pregnancy outcomes.[25-30] Selected saturated fatty acids included total SFA concentrations, myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0). Monounsaturated fatty acids included total MUFA concentrations, palmitoleic acid (C16:1n7) and oleic acid (C18:1n9). Polyunsaturated fatty acid included total n-3 PUFA concentrations, α-linolenic acid (ALA, C18:3n3), eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3), and total n-6 PUFA concentrations, linoleic acid (LA, C18:2n6), dihomo-γ-linolenic acid (DGLA, C20:3n6) and arachidonic acid (AA, C20:4n6). The ratio of total n-6/n-3 PUFA was calculated.[31]

Covariates

We obtained information on maternal prepregnancy body mass index, age, education level, ethnicity, parity, smoking, alcohol consumption and folic acid supplement by questionnaires.[21] First trimester maternal nutritional information was obtained by food frequency questionnaire.[32]

Statistical analysis

Differences in subject characteristics between body mass index categories were examined with 1-way ANOVA tests. We assessed the associations of prepregnancy body mass index, continuously and in categories, with fatty acid concentrations using linear regression models. Next, we assessed the associations of gestational weight gain, continuously and in categories, with fatty acid concentrations during pregnancy using linear regression models. To enable comparison of effect estimates, we constructed standard deviation scores (SDS) ((observed value – mean) / SD) for each fatty acid concentration. All models were adjusted for maternal age, educational level, ethnic-

ity, parity, smoking, alcohol consumption, folic acid supplement use and total caloric and fat intake during pregnancy. We have adjusted the regression models for these maternal lifestyle factors, as they are known to be related with the main exposures and outcomes. In addition, we also adjusted the analyses for folic acid supplement use, which is known to increase plasma concentrations of docosahexaenoic acid and eicosapentaenoic acid.[33] We performed sensitivity analyses without adjusting for total energy and fat intake. In order to test if the associations of gestational weight gain with plasma fatty acid concentrations are influenced by maternal prepregnancy body mass index, we presented results from the regression models unadjusted and adjusted for maternal prepregnancy body mass index. Also, we tested potential interactions between maternal prepregnancy body mass index categories and gestational weight gain for the associations with plasma fatty acid concentrations. Since we did not observe significant interactions, we did not perform the analyses focused on gestational weight gain effects in strata of maternal prepregnancy body mass index categories. Because of the correlation between the different fatty acids, we did not apply correction for multiple testing. In order to reduce potential bias associated with missing data and to maintain statistical power, we performed multiple imputations of missing covariates by generating 5 independent datasets using the Markov Chain Monte Carlo method after which the pooled effect estimates were calculated. All analyses were performed using Statistical Package of Social Sciences version 21.0 for Windows (SPSS Inc, Chicago, IL).

Results

Subject characteristics

Table 2.1.1 shows that obese pregnant women tended to be younger and lower educated, as compared to normal weight women. Obese women also tended to have a lower total energy intake and were more likely to have excessive weight gain during pregnancy, compared to normal weight women. Correlations between all fatty acid concentrations are shown in **Supplementary Table S2.1.1.** Observed mid-pregnancy fatty acid concentrations according to body mass index are shown in **Supplementary Tables S2.1.2. Table S2.1.3** shows characteristics of women according to gestational weight gain categories.

Table 2.1.1. Characteristics of mothers (N = 5,636) [a]

	Body mass categories					
	Total Group (5,636)	**Underweight [<20.0 kg/m²] (n=881)**	**Normal weight [20.0-24.9 kg/m²] (n=3,162)**	**Overweight [25.0-29.9 kg/m²] (n=1,094)**	**Obesity [≥30.0 kg/m²] (n=499)**	**P Value***
Maternal Characteristics						
Age (years)	29.8 (5.2)	29.2 (5.3)	30.1 (5.2)	29.8 (5.2)	29.2 (5.0)	<0.01
Height (cm)	167.6 (7.3)	168.7 (7.0)	167.9 (7.3)	166.3 (7.5)	165.7 (7.4)	<0.01
Weight (kg)	69.2 (13.1)	56.9 (6.7)	65.7 (7.3)	77.5 (8.6)	95.2 (13.8)	<0.01
Body mass index (kg/m²)	23.7 (4.4)	18.9 (0.9)	22.2 (1.4)	26.9 (1.4)	33.9 (3.7)	<0.01
Education, No. higher education (%)	2340 (42.9)	389 (45.3)	1508 (48.9)	360 (34.5)	83 (17.8)	<0.01
Race / ethnicity, No. European (%)	3259 (58.4)	544 (62.2)	1976 (63.0)	529 (49.1)	210 (42.9)	<0.01
Parity, No. nulliparous (%)	3230 (57.3)	563 (63.9)	1917 (60.7)	534 (48.8)	216 (43.3)	<0.01
Total energy intake (kcal)	2013 (587)	2061 (606)	2040 (574)	1953 (588)	1878 (602)	<0.01
Carbohydrates, energy (%)	49.1 (6.6)	49.3 (6.9)	49.3 (6.5)	48.8 (6.6)	48.1 (7.2)	0.06
Proteins, energy (%)	14.9 (2.7)	14.6 (2.8)	14.9 (2.6)	15.0 (2.7)	15.2 (3.1)	0.03
Fat, Energy%	35.8 (5.7)	35.9 (5.9)	35.6 (5.5)	36.0 (5.6)	36.6 (6.5)	0.07
Folic acid supplement use (yes), No. (%)	3354 (72.0)	535 (74.5)	1990 (66.9)	596 (66.0)	233 (68.6)	<0.01
Smoking during pregnancy (yes), No. (%)	1442 (26.7)	249 (29.4)	778 (25.6)	279 (26.9)	136 (28.5)	0.03
Alcohol consumption during pregnancy (yes), No. (%)	2785 (51.8)	478 (56.4)	1713 (56.5)	427 (41.6)	167 (35.5)	<0.01
Gestational weight gain (kg)	14.9 (5.7)	14.9 (5.0)	15.4 (5.1)	14.3 (6.4)	11.7 (8.1)	<0.01
Gestational weight gain categories						
Insufficient, N (%)	546 (20.2)	93 (21.2)	355 (21.6)	62 (13.6)	36 (22.8)	<0.01
Sufficient, N (%)	922 (34.2)	198 (45.1)	618 (37.6)	76 (16.7)	30 (19.0)	
Excessive, N (%)	1229 (45.6)	148 (33.7)	671 (40.8)	318 (69.7)	92 (58.2)	

[a]Values represent mean (SD), median (95% range), or number of subjects (valid%).
*Differences in subject characteristics between groups were evaluated using 1-way ANOVA test.

Saturated and monounsaturated fatty acid concentrations

Table 2.1.2 shows that a 1-SD higher body mass index was associated with 0.07 SD [95% confidence interval (CI): 0.04, 0.10] higher total SFA concentrations, and specifically with higher palmitic acid and stearic acid concentrations, and with lower myristic acid concentrations (all *P* values<0.05). As compared to normal weight women, overweight and obese women had higher total SFA concentrations (all *P* values<0.05). A 1-SD higher gestational weight gain was associated with 0.09 SD (95% CI: 0.05, 0.13) higher total SFA concentrations, and specifically with higher myristic, palmitic and stearic acid concentrations. Total saturated fatty acid concentrations

were not different between women with insufficient weight gain and with sufficient weight gain.

Table 2.1.3 shows that body mass index was not associated with total MUFA and oleic acid concentrations. However, a 1-SD higher body mass index was associated with 0.08 SD (95% CI: 0.06, 0.11) higher palmitoleic acid concentrations. A 1-SD higher gestational weight gain was associated with 0.10 SD (95% CI: 0.06, 0.13)

Table 2.1.2. Maternal weight during pregnancy with saturated fatty acid concentrations (N = 5,636)

	Difference in saturated fatty acid (SFA) concentrations (95% Confidence Interval)			
	Total SFAs (SD)	**Myristic acid (SD)**	**Palmitic acid (SD)**	**Stearic acid (SD)**
Body mass index[a]				
Underweight [<20.0 kg/m^2]	-0.18 (-0.25, -0.10)*	-0.03 (-0.10, 0.04)	-0.17 (-0.25, -0.10)*	-0.16 (-0.24, -0.09)*
Normal weight [20.0-24.9 kg/m^2]	*Reference*	*Reference*	*Reference*	*Reference*
Overweight [25.0-29.9 kg/m^2]	0.08 (0.01, 0.14)*	-0.13 (-0.19, -0.06)*	0.08 (0.01, 0.15)*	0.08 (0.02, 0.15)*
Obesity [≥30.0 kg/m^2]	0.10 (0.00, 0.19)*	-0.27 (-0.37, -0.18)*	0.13 (0.03, 0.22)*	0.07 (-0.03, 0.16)
Body mass index (SD)	0.07 (0.04, 0.10)*	-0.08 (-0.11, -0.06)*	0.08 (0.05, 0.11)*	0.06 (0.04. 0.09)*
	Difference in saturated fatty acid (SFA) concentrations (95% Confidence Interval)			
	Total SFAs (SD)	**Myristic acid (SD)**	**Palmitic acid (SD)**	**Stearic acid (SD)**
Gestational weight gain[b]				
Insufficient gestational weight gain	-0.06 (-0.16, 0.05)	-0.27 (-0.37, -0.17)*	-0.04 (-0.14, 0.06)	-0.06 (-0.16, 0.05)
Sufficient gestational weight gain	*Reference*	*Reference*	*Reference*	*Reference*
Excessive gestational weight gain	0.16 (0.08, 0.25)*	0.15 (0.07, 0.23)*	0.15 (0.06, 0.23)*	0.17 (0.09, 0.26)*
Gestational weight gain (SD)[c]	0.09 (0.05, 0.13)*	0.18 (0.14, 0.22)*	0.08 (0.04, 0.11)*	0.10 (0.06, 0.14)*
Gestational weight gain (SD)[d]	0.10 (0.06, 0.14)*	0.17 (0.13, 0.21)*	0.09 (0.05, 0.12)*	0.11 (0.07, 0.15)*

[a]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of saturated fatty acid concentrations for underweight, overweight and obese women as compared to normal weight women, and per SD increase in pre-pregnancy body mass index. Models are adjusted for age, educational level, ethnicity, parity, smoking and alcohol consumption, folic acid supplement use and total caloric and fat intake during pregnancy. [b]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of saturated fatty acid concentrations for insufficient weight gain and excessive weight gain women as compared to women with sufficient weight gain, and per SD increase in weight gain. Models are adjusted for age, educational level, ethnicity, parity, smoking and alcohol consumption, folic acid supplement use and total caloric and fat intake during pregnancy. Models for weight gain defined according to the IOM criteria are additionally adjusted for pre-pregnancy body mass index. [c]Gestational weight gain SD unadjusted for body mass index. [d]Gestational weight gain SD adjusted for body mass index. * P-value<0.05

higher total MUFA concentrations and all specific MUFA concentrations. Compared to women with sufficient weight gain, those with excessive weight gain had higher total MUFA, palmitoleic and oleic acid concentrations (all *P* values<0.05).

N-3 and n-6 polyunsaturated fatty acid concentrations

Table 2.1.4 shows that higher body mass index was not associated with total n-3 PUFA and docosahexaenoic acid concentrations, but was associated with lower a-linolenic acid and eicosapentaenoic acid concentrations (all *P* values<0.05). As compared to normal weight women, underweight women tended to have lower

Table 2.1.3. Maternal weight during pregnancy with monounsaturated fatty acid concentrations (N = 5,636)

	Difference in monounsaturated fatty acids (MUFAs) concentrations (95% Confidence Interval)		
	Total MUFAs (SD)	**Palmitoleic acid (SD)**	**Oleic acid (SD)**
Body mass index[a]			
Underweight [<20.0 kg/m^2]	-0.05 (-0.12, 0.02)	-0.12 (-0.19, -0.05)*	-0.03 (-0.10, 0.04)
Normal weight [20.0-24.9 kg/m^2]	*Reference*	*Reference*	*Reference*
Overweight [25.0-29.9 kg/m^2]	-0.02 (-0.08, 0.05)	0.06 (-0.01, 0.13)	-0.03 (-0.10, 0.04)
Obesity [≥30.0 kg/m^2]	-0.02 (-0.11, 0.07)	0.22 (0.13, 0.32)*	-0.07 (-0.16, 0.02)
Body mass index (SD)	0 (-0.02, 0.03)	0.08 (0.06, 0.11)*	-0.01 (-0.04, 0.01)
	Difference in monounsaturated fatty acids (MUFAs) concentrations (95% Confidence Interval)		
	Total MUFAs (SD)	**Palmitoleic acid (SD)**	**Oleic acid (SD)**
Gestational weight gain[b]			
Insufficient gestational weight gain	-0.04 (-0.14, 0.06)	-0.12 (-0.22, -0.01)*	-0.04 (-0.14, 0.06)
Sufficient gestational weight gain	*Reference*	*Reference*	*Reference*
Excessive gestational weight gain	0.16 (0.08, 0.24)*	0.18 (0.10, 0.27)*	0.17 (0.08, 0.24)*
Gestational weight gain (SD)[c]	0.10 (0.06, 0.13)*	0.13 (0.10, 0.17)*	0.10 (0.06, 0.14)*
Gestational weight gain (SD)[d]	0.10 (0.06, 0.14)*	0.15 (0.11, 0.19)*	0.10 (0.06, 0.14)*

[a]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of monounsaturated fatty acid concentrations for underweight, overweight and obese women as compared to normal weight women, and per SD increase in pre-pregnancy body mass index. Models are adjusted for age, educational level, ethnicity, parity, smoking and alcohol consumption, folic acid supplement use and total caloric and fat intake during pregnancy. [b]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of monounsaturated fatty acid concentrations for insufficient weight gain and excessive weight gain women as compared to women with sufficient weight gain, and per SD increase in weight gain. Models are adjusted for age, educational level, ethnicity, parity, smoking and alcohol consumption, folic acid supplement use and total caloric and fat intake during pregnancy. Models for weight gain defined according to the IOM criteria are additionally adjusted for pre-pregnancy body mass index. [c]Gestational weight gain SD unadjusted for body mass index. [d]Gestational weight gain SD adjusted for body mass index. * P-value<0.05

total n-3 PUFA and all specific n-3 PUFA concentrations (all *P* values<0.05). Higher gestational weight gain was not associated with n-3 PUFA and docosahexaenoic acid concentrations, but was associated with higher a-linolenic acid eicosapentaenoic acid concentrations. None of the n-3 PUFA concentrations were different between women with excessive weight gain as compared to those with sufficient weight gain.

Table 2.1.4. Maternal weight during pregnancy with n-3 polyunsaturated fatty acid concentrations (N = 5,636)

	Difference in n-3 Polyunsaturated fatty acids (PUFAs) concentrations (95% Confidence Interval)			
	Total n-3 PUFAs (SD)	**α-Linolenic acid (SD)**	**Eicosapentaenoic acid (SD)**	**Docosahexaenoic acid (SD)**
Body mass index[a]				
Underweight [<20.0 kg/m^2]	-0.11 (-0.18, -0.05)*	0.06 (-0.01, 0.13)	-0.03 (-0.10, 0.04)	-0.13 (-0.20, -0.06)*
Normal weight [20.0-24.9 kg/m^2]	*Reference*	*Reference*	*Reference*	*Reference*
Overweight [25.0-29.9 kg/m^2]	-0.04 (-0.11, 0.02)	-0.17 (-0.24, -0.11)*	-0.05 (-0.11, 0.02)	-0.01 (-0.07, 0.06)
Obesity [≥30.0 kg/m^2]	-0.11 (-0.20, -0.02)*	-0.32 (-0.41, -0.23)*	-0.10 (-0.19, -0.01)*	-0.05 (-0.14, 0.04)
Body mass index (SD)	-0.02 (-0.04, 0.01)	-0.12 (-0.15, -0.09)*	-0.03 (-0.05, -0.01)*	0.01 (-0.02, 0.03)
	Difference in n-3 Polyunsaturated fatty acids (PUFAs) concentrations (95% Confidence Interval)			
	Total n-3 PUFAs (SD)	**α-Linolenic acid (SD)**	**Eicosapentaenoic acid (SD)**	**Docosahexaenoic acid (SD)**
Gestational weight gain[b]				
Insufficient gestational weight gain	-0.07 (-0.17, 0.03)	-0.14 (-0.24, -0.04)*	-0.18 (-0.28, -0.07)*	-0.01 (-0.11, 0.09)
Sufficient gestational weight gain	*Reference*	*Reference*	*Reference*	*Reference*
Excessive gestational weight gain	0.01 (-0.08, 0.09)	0.05 (-0.03, 0.14)	0.02 (-0.07, 0.11)	-0.02 (-0.11, 0.06)
Gestational weight gain (SD)[c]	0.03 (-0.01, 0.06)	0.09 (0.05, 0.13)*	0.08 (0.04, 0.12)*	-0.02 (-0.06, 0.02)
Gestational weight gain (SD)[d]	0.02 (-0.02, 0.06)	0.07 (0.04, 0.11)*	0.08 (0.04, 0.12)*	-0.02 (-0.06, 0.02)

[a]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of n-3 PUFA concentrations for underweight, overweight and obese women as compared to normal weight women, and per SD increase in pre-pregnancy body mass index. Models are adjusted for, age, educational level, ethnicity, parity, smoking and alcohol consumption, folic acid supplement use and total caloric and fat intake during pregnancy. [b]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of n-3 PUFA concentrations for insufficient weight gain and excessive weight gain women as compared to women with sufficient weight gain, and per SD increase in weight gain. Models are adjusted for, age, educational level, ethnicity, parity, smoking and alcohol consumption, folic acid supplement use and total caloric and fat intake during pregnancy. Models for weight gain defined according to the IOM criteria are additionally adjusted for pre-pregnancy body mass index. [c]Gestational weight gain SD unadjusted for body mass index. [d]Gestational weight gain SD adjusted for body mass index. *P-value<0.05

Table 2.1.5 shows that 1-SD higher body mass index was associated with 0.06 SD (95% CI: 0, 0.09) higher total n-6 PUFAs, and specifically with higher dihomo-γ-linoelinic acid and arachidonic acid, but with lower linoleic acid concentrations (all P values<0.05). Compared to normal weight women, obese women had lower linoleic acid concentrations, but higher dihomo-γ-linolenic and arachidonic acid concentrations (all *P* values<0.05). A 1-SD higher gestational weight gain was associated with 0.04 SD (95% CI: 0.01, 0.08) higher total n-6 PUFAs and specifically with higher dihomo-γ-linoelinic acid concentrations. As compared to women with sufficient weight gain, those with excessive weight gain had higher n-6 PUFA concentrations (*P* values<0.05). Only linoleic acid concentrations were not different between normal weight and obese women.

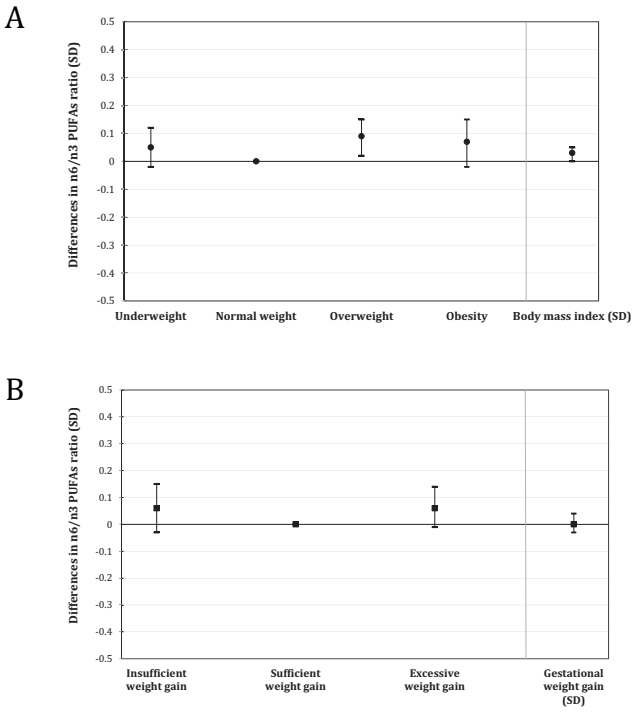
Table 2.1.5. Maternal weight during pregnancy with n-6 polyunsaturated fatty acid concentrations (N = 5,636)

	Difference in n-6 Polyunsaturated fatty acids (PUFAs) concentrations (95% Confidence Interval)			
	Total n-6 PUFAs (SD)	**Linoleic acid (SD)**	**Dihomo-γ-linolenic acid (SD)**	**Arachidonic acid (SD)**
Body mass index[a]				
Underweight [<20.0 kg/m^2]	-0.17 (-0.24, -0.09)*	-0.03 (-0.11, 0.04)	-0.27 (-0.34, -0.20)*	-0.23 (-0.30, -0.16)*
Normal weight [20.0-24.9 kg/m^2]	*Reference*	*Reference*	*Reference*	*Reference*
Overweight [25.0-29.9 kg/m^2]	0.09 (0.02, 0.16)*	-0.03 (-0.09, 0.04)	0.16 (0.10, 0.23)*	0.23 (0.16, 0.30)*
Obesity [≥30.0 kg/m^2]	0.06 (-0.04, 0.15)	-0.23 (-0.32, -0.13)*	0.35 (0.26, 0.45)*	0.45 (0.35, 0.54)*
Body mass index (SD)	0.06 (0, 0.09)*	-0.05 (-0.08, -0.02)*	0.16 (0.14, 0.19)*	0.19 (0.17, 0.22)*
	Difference in n-6 Polyunsaturated fatty acids (PUFAs) concentrations (95% Confidence Interval)			
	Total n-6 PUFAs (SD)	**Linoleic acid (SD)**	**Dihomo-γ-linolenic acid (SD)**	**Arachidonic acid (SD)**
Gestational weight gain[b]				
Insufficient gestational weight gain	0.02 (-0.08, 0.12)	0.02 (-0.09, 0.12)	-0.08 (-0.18, 0.02)	0.07 (-0.04, 0.17)
Sufficient gestational weight gain	*Reference*	*Reference*	*Reference*	*Reference*
Excessive gestational weight gain	0.12 (0.04, 0.21)*	0.05 (-0.04, 0.13)	0.29 (0.21, 0.37)*	0.05 (-0.03, 0.13)
Gestational weight gain (SD)[c]	0.04 (0.01, 0.08)*	0.02 (-0.02, 0.06)	0.15 (0.11, 0.19)*	-0.03 (-0.06, 0.01)
Gestational weight gain (SD)[d]	0.05 (0.02, 0.09)*	0.01 (-0.02, 0.05)	0.18 (0.14, 0.22)*	-0.00 (-0.04, 0.04)

[a]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of n-6 PUFA concentrations for underweight, overweight and obese women as compared to normal weight women, and per SD increase in pre-pregnancy body mass index. Models are adjusted for age, educational level, ethnicity, parity, smoking and alcohol consumption, folic acid supplement use and total caloric and fat intake during pregnancy. [b]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of n-6 PUFA concentrations for insufficient weight gain and excessive weight gain women as compared to women with sufficient weight gain, and per SD increase in weight gain. Models are adjusted for age, educational level, ethnicity, parity, smoking and alcohol consumption, folic acid supplement use and total caloric and fat intake during pregnancy. Models for weight gain defined according to the IOM criteria are additionally adjusted for pre-pregnancy body mass index. [c]Gestational weight gain SD unadjusted for body mass index. [d]Gestational weight gain SD adjusted for body mass index. *P-value<0.05

Figure 2.1.2 shows that as compared to normal weight women, those with obesity tended to have a 0.07 SD (95% CI: - 0.02, 0.15) higher n-6/n-3 PUFA ratio. Also, as compared to women with sufficient weight gain, those with excessive weight gain had a 0.06 SD (95% CI: - 0.01, 0.14) higher n-6/n-3 PUFA ratio.

Figure 2.1.2. Maternal body mass index (A) and gestational weight gain (B) with n-6/n-3 PUFA ratio (N = 5,636)[a]



[a] (A) Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of n-6/n-3 PUFAs ratio for underweight, overweight and obese women as compared to normal weight women, and per SD increase in pre-pregnancy body mass index. Models are adjusted for maternal age, educational level, ethnicity, parity, smoking and alcohol consumption, folic acid supplement use and total caloric and fat intake during pregnancy. * P-value<0.05. (B) Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of n-6/n-3 PUFAs ratio for insufficient weight gain, and excessive weight gain women as compared to women with sufficient weight gain, and per SD increase in weight gain. Models are adjusted for age, educational level, ethnicity, parity, smoking and alcohol consumption, folic acid supplement use and total caloric and fat intake during pregnancy. Models for weight gain are additionally adjusted for pre-pregnancy body mass index. * P-value<0.05.

Additional analysis

Similar results were observed when we did not adjust the gestational weight gain analyses for maternal prepregnancy body mass index (Results given in **Supplementary Tables S2.1.4 and S2.1.5**). We observed similar results for all analyses without adjustment for total energy and fat intake (**Supplementary Tables S2.1.6–S2.1.9**). The results from the unadjusted models are given in **Supplementary Tables S2.1.10–S2.1.13**. Results were not materially different between the adjusted and unadjusted models.

Discussion

In this population-based cohort study, we observed that higher prepregnancy body mass index was associated with higher total SFA and total n-6 PUFA concentrations. Also, we observed that higher gestational weight gain was, independent of pre-pregnancy body mass index, associated with higher total SFA, MUFA and n-6 PUFA concentrations.

Methodological considerations

This study was embedded in a population-based prospective cohort with a large number of subjects. To the best of our knowledge, the current study is the largest population-based study focused on the associations of body mass index and weight gain during gestation with fatty acid concentrations. However, some limitations need to be discussed. Within this study, we correlated prepregnancy body mass index and maximum weight gain with mid-pregnancy fatty acid concentrations. The timing of these measurements restricts us for drawing conclusions about the direction of any association. However, fatty acid patterns in plasma phospholipids show a relatively high degree of tracking over time.[34,35] Information on maternal prepregnancy weight and maximum weight was self-reported. We observed high correlations between weight in early and late pregnancy with prepregnancy weight and maximum weight, respectively, but self-reported weight might be underestimated. We measured a large number of fatty acid concentrations in plasma samples only once during pregnancy. No information was available about fatty acid concentrations in other trimesters. Fatty acids measured in plasma may reflect a time frame of dietary intake of approximately 2 weeks and seem to be reasonable indicators for the recent intake [36]. Unfortunately, no information was available about erythrocyte lipid levels, which would reflect a longer intake period. We assessed maternal dietary intake during first trimester by a food frequency questionnaire (FFQ) and adjusted the main analyses for maternal total energy and fat intake. No differences in results were observed with and without adjustment for total energy and fat intake. Unfortunately, these FFQs were not validated for assessing maternal dietary fatty acids intake. Further observational and experimental studies are needed to explore the associations of maternal dietary fatty acids intake with maternal body mass index and gestational weight gain. Although, we were able to adjust our analyses for various potential confounders, residual confounding might still be an issue as in any observational study.

Interpretation of main findings

Overweight and obesity during pregnancy are important public health problems and are associated with adverse maternal and neonatal outcomes.[9,37] Multiple previous studies have reported that higher prepregnancy body mass index and excessive gestational weight gain are associated with increased risks of gestational diabetes, neonatal mortality, and obesity in offspring.[11,12,15,37-39] The mechanism linking higher maternal obesity and excessive gestational weight gain with offspring outcomes may include fetal overnutrition.[16] Fetal exposure to increased concentrations of free fatty acids may lead to intrauterine metabolic adaptations and disproportionate fetal growth.[40] Increased concentrations of free fatty acids during pregnancy are associated with childhood obesity.[41,42] Previously, we have shown that higher maternal n-3 PUFA and lower n-6 PUFA concentrations in pregnancy are also associated with lower childhood systolic blood pressure.[43]

Results from previous studies support associations of obesity with fatty acid concentrations. A study among 124 adults in Australia showed that obese subjects had lower plasma n-3 PUFA concentrations.[19] A systematic review based on 21 studies showed that overweight or obese adults had lower linoleic acid and higher dihomo-γ-linolenic acid, n-6 PUFAs.[44] A study among 120 adolescents aged 12 years old from France showed that overweight adolescents had higher SFA concentrations and lower total n-3 PUFA concentrations.[45] Only one study has assessed the associations of body mass index with fatty acid concentrations among pregnant women.[20] This study among 129 pregnant women in the US showed that as compared with lean women, obese women had lower docosahexaenoic acid, an n-3 PUFA, and arachidonic, an n-6 PUFA, concentrations during pregnancy.[20] No information about other fatty acid concentrations was available. In line with these previous results, we observed that higher body mass index was associated with higher total n-6 PUFA concentrations. Obese women had lower linoleic acid concentrations, but higher dihomo-γ-linolenic and arachidonic acid concentrations. All n-3 PUFA concentrations tended to be lower among obese women with the strongest association for α-linolenic acid. For the SFAs, obese women had lower myristic acid concentrations and higher palmitic acid concentrations, whereas for the MUFAs, obese women had higher palmitoleic acid concentrations. These results suggest that the associations of body mass index with fatty acid concentrations in pregnant women are in line with those observed in non-pregnant adults.

We also assessed the associations of gestational weight gain with fatty acid concentrations. Gestational weight gain is a complex measure, which reflects maternal fat accumulation, fluid and blood volume expansion, and fetal and placental growth.[23] Excessive gestational weight gain is defined as increased weight gain between the

time of conception and the onset of labor and is a related with pregnancy and offspring outcomes.[14] We observed that total SFA concentrations, specifically myristic, palmitic and stearic acids, were higher among women with excessive gestational weight gain. Excessive gestational weight gain was also associated with total MUFA concentrations, specifically palmitoleic and oleic acids. We observed that excessive gestational weight gain was associated with higher concentrations of total n-6 PUFA, specifically dihomo-γ-linoleic and arachidonic acids, but no association was observed with n-3 PUFA concentrations. The observed associations of excessive gestational weight gain with fatty acid concentrations were present with and without adjustment of body mass index. Thus, women with excessive gestational weight gain during pregnancy have independent of their prepregnancy body mass index, an adverse fatty acid profile.

Our results suggest that pregnant women with either a high body mass index or high gestational weight gain have an adverse fatty acids profile, characterized by higher concentrations of SFA, MUFA and n-6 PUFA, and lower concentrations of n-3 PUFA. From our study, it is difficult to determine whether body mass index and gestational weight gain cause adverse fatty acid profiles, or whether the direction of the association is reversed. It has been suggested that higher n-6 PUFA concentrations lead to higher adipose tissue development through promotion of preadipocyte differentiation and that higher n-3 PUFA concentrations decreases adipose tissue mass and suppresses development of obesity.[46,47] Also, saturated fatty acids and the monounsaturated fatty acids stimulate adipocyte differentiation and triacylglycerol accumulation.[48] In the other direction, obesity alters adipose tissue metabolic and endocrine function and leads to an increased release of fatty acids, affecting subsequent adiposity and thus creating a vicious cycle.[49] Further studies are needed to explore the causality and direction of the observed associations. Studies with longitudinal measurements of both weight and fatty acid concentrations before and during pregnancy may help to identify the direction of these associations. Also, experimental and Mendelian Randomization studies may help to assess the causality of the observed associations.[50,51]

Conclusion

We observed that higher prepregnancy body mass index was associated with higher total SFA and total n-6 PUFA concentrations, whereas higher gestational weight gain was associated with higher total SFA, MUFA and n-6 PUFA concentrations. Further

observational and experimental studies are needed for replication and to explore the direction and underlying mechanism of these associations.

References

1. Haslam DW, James WP. Obesity. Lancet 2005;366(9492):1197-1209.
2. Liu XM, Liu YJ, Zhan J, He QQ. Overweight, obesity and risk of all-cause and cardiovascular mortality in patients with type 2 diabetes mellitus: a dose-response meta-analysis of prospective cohort studies. Eur J Epidemiol 2015;30(1):35-45.
3. O'Doherty MG, Jorgensen T, Borglykke A, Brenner H, Schottker B, Wilsgaard T, Siganos G, Kavousi M, Hughes M, Muezzinler A, Holleczek B, Franco OH, Hofman A, Boffetta P, Trichopoulou A, Kee F. Repeated measures of body mass index and C-reactive protein in relation to all-cause mortality and cardiovascular disease: results from the consortium on health and ageing network of cohorts in Europe and the United States (CHANCES). Eur J Epidemiol 2014;29(12):887-897.
4. Niedziela J, Hudzik B, Niedziela N, Gasior M, Gierlotka M, Wasilewski J, Myrda K, Lekston A, Polonski L, Rozentryt P. The obesity paradox in acute coronary syndrome: a meta-analysis. Eur J Epidemiol 2014;29(11):801-812.
5. Horvei LD, Braekkan SK, Mathiesen EB, Njolstad I, Wilsgaard T, Hansen JB. Obesity measures and risk of venous thromboembolism and myocardial infarction. Eur J Epidemiol 2014;29(11):821-830.
6. Song X, Pukkala E, Dyba T, Tuomilehto J, Moltchanov V, Mannisto S, Jousilahti P, Qiao Q. Body mass index and cancer incidence: the FINRISK study. Eur J Epidemiol 2014;29(7):477-487.
7. Etemadi A, Abnet CC, Kamangar F, Islami F, Khademi H, Pourshams A, Poustchi H, Bagheri M, Sohrabpour AA, Aliasgar A, Khoshnia M, Wacholder S, Matthews CC, Pharoah PD, Brennan P, Boffetta P, Malekzadeh R, Dawsey SM. Impact of body size and physical activity during adolescence and adult life on overall and cause-specific mortality in a large cohort study from Iran. Eur J Epidemiol 2014;29(2):95-109.
8. Hjellvik V, Selmer R, Gjessing HK, Tverdal A, Vollset SE. Body mass index, smoking, and risk of death between 40 and 70 years of age in a Norwegian cohort of 32,727 women and 33,475 men. Eur J Epidemiol 2013;28(1):35-43.
9. Huda SS, Brodie LE, Sattar N. Obesity in pregnancy: prevalence and metabolic consequences. Semin Fetal Neonatal Med 2010;15(2):70-76.
10. Callaway LK, Prins JB, Chang AM, McIntyre HD. The prevalence and impact of overweight and obesity in an Australian obstetric population. Med J Aust 2006;184(2):56-59.
11. Starling AP, Brinton JT, Glueck DH, Shapiro AL, Harrod CS, Lynch AM, Siega-Riz AM, Dabelea D. Associations of maternal BMI and gestational weight gain with neonatal adiposity in the Healthy Start study. Am J Clin Nutr 2015;101(2):302-309.
12. Kongubol A, Phupong V. Prepregnancy obesity and the risk of gestational diabetes mellitus. BMC Pregnancy Childbirth 2011;11:59.
13. Driul L, Cacciaguerra G, Citossi A, Martina MD, Peressini L, Marchesoni D. Prepregnancy body mass index and adverse pregnancy outcomes. Arch Gynecol Obstet 2008;278(1):23-26.
14. Gaillard R. Maternal obesity during pregnancy and cardiovascular development and disease in the offspring. Eur J Epidemiol 2015.

15. Gaillard R, Steegers EA, Franco OH, Hofman A, Jaddoe VW. Maternal weight gain in different periods of pregnancy and childhood cardio-metabolic outcomes. The Generation R Study. Int J Obes (Lond) 2015;39(4):677-685.
16. Lawlor DA, Smith GD, O'Callaghan M, Alati R, Mamun AA, Williams GM, Najman JM. Epidemiologic evidence for the fetal overnutrition hypothesis: findings from the mater-university study of pregnancy and its outcomes. Am J Epidemiol 2007;165(4):418-424.
17. Nelson SM, Matthews P, Poston L. Maternal metabolism and obesity: modifiable determinants of pregnancy outcome. Hum Reprod Update 2010;16(3):255-275.
18. Karlsson M, Marild S, Brandberg J, Lonn L, Friberg P, Strandvik B. Serum phospholipid fatty acids, adipose tissue, and metabolic markers in obese adolescents. Obesity (Silver Spring) 2006;14(11):1931-1939.
19. Micallef M, Munro I, Phang M, Garg M. Plasma n-3 Polyunsaturated Fatty Acids are negatively associated with obesity. Br J Nutr 2009;102(9):1370-1374.
20. Tomedi LE, Chang CC, Newby PK, Evans RW, Luther JF, Wisner KL, Bodnar LM. Pre-pregnancy obesity and maternal nutritional biomarker status during pregnancy: a factor analysis. Public Health Nutr 2013;16(8):1414-1418.
21. Jaddoe VW, van Duijn CM, Franco OH, van der Heijden AJ, van Iizendoorn MH, de Jongste JC, van der Lugt A, Mackenbach JP, Moll HA, Raat H, Rivadeneira F, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC, Hofman A. The Generation R Study: design and cohort update 2012. Eur J Epidemiol 2012;27(9):739-756.
22. Kruithof CJ, Kooijman MN, van Duijn CM, Franco OH, de Jongste JC, Klaver CC, Mackenbach JP, Moll HA, Raat H, Rings EH, Rivadeneira F, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC, Wolvius EB, Hofman A, Jaddoe VW. The Generation R Study: Biobank update 2015. Eur J Epidemiol 2014;29(12):911-927.
23. Guidelines IoMUaNRCUCtRIPW. Weight Gain During Pregnancy: Reexamining the Guidelines. In: Rasmussen KM YA, editors., editor. 2010/07/30 ed. Washington, DC, USA: National Academies Press; 2009.
24. Glaser C, Demmelmair H, Koletzko B. High-throughput analysis of fatty acid composition of plasma glycerophospholipids. J Lipid Res 2010;51(1):216-221.
25. Wang L, Manson JE, Rautiainen S, Gaziano JM, Buring JE, Tsai MY, Sesso HD. A prospective study of erythrocyte polyunsaturated fatty acid, weight gain, and risk of becoming overweight or obese in middle-aged and older women. Eur J Nutr 2015.
26. Kris-Etherton PM. AHA Science Advisory. Monounsaturated fatty acids and risk of cardiovascular disease. American Heart Association. Nutrition Committee. Circulation 1999;100(11):1253-1258.
27. Markey O, Vasilopoulou D, Givens DI, Lovegrove JA. Dairy and cardiovascular health: Friend or foe? Nutr Bull 2014;39(2):161-171.
28. Chen X, Scholl TO, Leskiw M, Savaille J, Stein TP. Differences in maternal circulating fatty acid composition and dietary fat intake in women with gestational diabetes mellitus or mild gestational hyperglycemia. Diabetes Care 2010;33(9):2049-2054.
29. Jensen CL. Effects of n-3 fatty acids during pregnancy and lactation. Am J Clin Nutr 2006;83(6 Suppl):1452S-1457S.
30. Qiu C, Sanchez SE, Larrabure G, David R, Bralley JA, Williams MA. Erythrocyte omega-3 and omega-6 polyunsaturated fatty acids and preeclampsia risk in Peruvian women. Arch Gynecol Obstet 2006;274(2):97-103.

31. Steenweg-de Graaff JC, Tiemeier H, Basten MG, Rijlaarsdam J, Demmelmair H, Koletzko B, Hofman A, Jaddoe VW, Verhulst FC, Roza SJ. Maternal LC-PUFA status during pregnancy and child problem behavior: the Generation R Study. Pediatr Res 2015;77(3):489-497.
32. Heppe DH, Medina-Gomez C, Hofman A, Franco OH, Rivadeneira F, Jaddoe VW. Maternal first-trimester diet and childhood bone mass: the Generation R Study. Am J Clin Nutr 2013;98(1):224-232.
33. Das UN. Folic acid and polyunsaturated fatty acids improve cognitive function and prevent depression, dementia, and Alzheimer's disease--but how and why? Prostaglandins Leukot Essent Fatty Acids 2008;78(1):11-19.
34. Zeleniuch-Jacquotte A, Chajes V, Van Kappel AL, Riboli E, Toniolo P. Reliability of fatty acid composition in human serum phospholipids. Eur J Clin Nutr 2000;54(5):367-372.
35. Guerra A, Demmelmair H, Toschke AM, Koletzko B. Three-year tracking of fatty acid composition of plasma phospholipids in healthy children. Ann Nutr Metab 2007;51(5):433-438.
36. Skeaff CM, Hodson L, McKenzie JE. Dietary-induced changes in fatty acid composition of human plasma, platelet, and erythrocyte lipids follow a similar time course. J Nutr 2006;136(3):565-569.
37. Gaillard R, Felix JF, Duijts L, Jaddoe VW. Childhood consequences of maternal obesity and excessive weight gain during pregnancy. Acta Obstet Gynecol Scand 2014;93(11):1085-1089.
38. Johansson S, Villamor E, Altman M, Bonamy AK, Granath F, Cnattingius S. Maternal overweight and obesity in early pregnancy and risk of infant mortality: a population based cohort study in Sweden. BMJ 2014;349:g6572.
39. Lau EY, Liu J, Archer E, McDonald SM, Liu J. Maternal weight gain in pregnancy and risk of obesity among offspring: a systematic review. J Obes 2014;2014:524939.
40. Schaefer-Graf UM, Graf K, Kulbacka I, Kjos SL, Dudenhausen J, Vetter K, Herrera E. Maternal lipids as strong determinants of fetal environment and growth in pregnancies with gestational diabetes mellitus. Diabetes Care 2008;31(9):1858-1863.
41. Poston L. Maternal obesity, gestational weight gain and diet as determinants of offspring long term health. Best Pract Res Clin Endocrinol Metab 2012;26(5):627-639.
42. Moon RJ, Harvey NC, Robinson SM, Ntani G, Davies JH, Inskip HM, Godfrey KM, Dennison EM, Calder PC, Cooper C, Group SWSS. Maternal plasma polyunsaturated fatty acid status in late pregnancy is associated with offspring body composition in childhood. J Clin Endocrinol Metab 2013;98(1):299-307.
43. Vidakovic AJ, Gishti O, Steenweg-de Graaff J, Williams MA, Duijts L, Felix JF, Hofman A, Tiemeier H, Jaddoe VW, Gaillard R. Higher Maternal Plasma n-3 PUFA and Lower n-6 PUFA Concentrations in Pregnancy Are Associated with Lower Childhood Systolic Blood Pressure. J Nutr 2015;145(10):2362-2368.
44. Fekete K, Gyorei E, Lohner S, Verduci E, Agostoni C, Decsi T. Long-chain polyunsaturated fatty acid status in obesity: a systematic review and meta-analysis. Obesity reviews : an official journal of the International Association for the Study of Obesity 2015.
45. Klein-Platat C, Drai J, Oujaa M, Schlienger JL, Simon C. Plasma fatty acid composition is associated with the metabolic syndrome and low-grade inflammation in overweight adolescents. Am J Clin Nutr 2005;82(6):1178-1184.
46. Buckley JD, Howe PR. Anti-obesity effects of long-chain omega-3 polyunsaturated fatty acids. Obes Rev 2009;10(6):648-659.
47. Muhlhausler BS, Ailhaud GP. Omega-6 polyunsaturated fatty acids and the early origins of obesity. Curr Opin Endocrinol Diabetes Obes 2013;20(1):56-61.

48. Madsen L, Petersen RK, Kristiansen K. Regulation of adipocyte differentiation and function by polyunsaturated fatty acids. Biochim Biophys Acta 2005;1740(2):266-286.
49. Kishino T, Watanabe K, Urata T, Takano M, Uemura T, Nishikawa K, Mine Y, Matsumoto M, Ohtsuka K, Ohnishi H, Mori H, Takahashi S, Ishida H, Watanabe T. Visceral fat thickness in overweight men correlates with alterations in serum fatty acid composition. Clin Chim Acta 2008;398(1-2):57-62.
50. Smith GD, Ebrahim S. Mendelian randomization: prospects, potentials, and limitations. Int J Epidemiol 2004;33(1):30-42.
51. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol 2003;32(1):1-22.

Supplemental Material

Table S2.1.1. Correlation between all maternal fatty acid concentrations[a]

Fatty acids	Total SFA	14:0	16:0	18:0	Total MUFAs	16:1n7	18:1n9	Total n-3 PUFA	18:3n3	20:5n3	22:6n3	Total n-6 PUFA	18:2n6	20:3n6	20:4n6
Total SFA	1														
14:0	0.59*	1													
16:0	0.98*	0.59*	1												
18:0	0.87*	0.41*	0.78*	1											
Total MUFA	0.79*	0.57*	0.81*	0.60*	1										
16:1n7	0.60*	0.63*	0.65*	0.33*	0.73*	1									
18:1n9	0.77*	0.55*	0.78*	0.60*	0.99*	0.66*	1								
Total n-3 PUFA	0.51*	0.28*	0.51*	0.42*	0.45*	0.26*	0.43*	1							
18:3n3	0.44*	0.44*	0.44*	0.35*	0.52*	0.35*	0.52*	0.34*	1						
20:5n3	0.28*	0.24*	0.29*	0.20*	0.29*	0.21*	0.28*	0.77*	0.28*	1					
22:6n3	0.46*	0.20*	0.47*	0.39*	0.38*	0.18*	0.36*	0.97*	0.21*	0.64*	1				
Total n-6 PUFA	0.85*	0.34*	0.80*	0.86*	0.50*	0.28*	0.50*	0.22*	0.29*	0.00	0.21*	1			
18:2n6	0.64*	0.23*	0.59*	0.69*	0.32*	0.05*	0.33*	0.08*	0.31*	-0.07*	0.07*	0.87*	1		
20:3n6	0.74*	0.52*	0.75*	0.59*	0.65*	0.66*	0.62*	0.23*	0.32*	0.11*	0.18*	0.61*	0.35*	1	
20:4n6	0.59*	0.14*	0.57*	0.59*	0.34*	0.23*	0.31*	0.32*	-0.01	0.11*	0.35*	0.63*	0.22*	0.35*	1

[a]Values are Pearson correlation coefficients. *P-value <0.06.

Table S2.1.2. Maternal mid-pregnancy fatty acid concentrations according to body mass index (N=5,636)[a]

	Total Group (5,636)	Underweight [<20.0 kg/m^2] (n=881)	Normal weight [20.0-24.9 kg/m^2] (n=3,162)	Overweight [25.0-29.9 kg/m^2] (n=1,094)	Obesity [≥30.0 kg/m^2] (n=499)	P-value*
Saturated fatty acids (SF)						
Total SFA, mg/L	696.3 (102.4)	677.8 (98.4)	698.5 (102.4)	702.6 (103.4)	701.1 (103.9)	<0.01
Myristic acid (C14:0), mg/L	10.6 (3.6)	10.7 (3.7)	10.9 (3.6)	10.2 (3.4)	9.5 (3.5)	<0.01
Palmitic acid (C16:0), mg/L	494.9 (76.6)	481.3 (74.0)	496.7 (76.6)	499.1 (77.5)	499.3 (77.1)	<0.01
Stearic acid (C18:0), mg/L	184.9 (27.8)	180.1 (26.0)	185.1 (27.8)	187.6 (28.7)	186.9 (28.2)	<0.01
Monounsaturated fatty acids (MUFAs)						
Total MUFA, mg/L	206.4 (40.1)	205.8 (39.8)	208.7 (39.8)	203.1 (40.7)	199.9 (40.2)	<0.01
Palmitoleic acid (C16:1n7), mg/L	12.1 (5.5)	11.5 (5.5)	12.2 (5.6)	12.1 (5.4)	12.8 (5.6)	<0.01
Oleic acid (C18:1n9), mg/L	23.7 (4.5)	23.3 (4.3)	23.9 (4.4)	23.5 (4.5)	23.6 (4.7)	<0.01
n-3 Polyunsaturated fatty acids (PUFAs)						
Total n-3 PUFAs, mg/L	103.8 (27.4)	101.7 (28.5)	106.3 (27.7)	101.4 (27.7)	96.9 (22.0)	<0.01
α-Linolenic acid (C18:3n3), mg/L	5.1 (1.9)	5.3 (2.0)	5.2 (1.8)	4.7 (1.8)	4.4 (1.6)	<0.01
Eicosapentaenoic acid (C20:5n3), mg/L	8.5 (5.4)	8.5 (5.7)	8.9 (5.5)	8.0 (5.1)	7.3 (3.9)	<0.01
Docosahexaenoic acid (C22:6n3), mg/L	76.7 (20.3)	74.5 (21.1)	78.2 (20.5)	75.7 (20.1)	72.9 (16.8)	<0.01
n-6 Polyunsaturated fatty acids (PUFAs)						
Total n-6 PUFA, mg/L	602.0 (88.9)	584.7 (82.8)	600.7 (88.9)	614.6 (90.9)	613.2 (89.5)	<0.01
Linoleic acid (C18:2n6), mg/L	360.6 (62.5)	358.4 (59.3)	361.2 (62.7)	364.1 (64.6)	353.1 (60.9)	<0.01
Dihomo-γ-linolenic acid (C20:3n6), mg/L	60.7 (16.8)	55.9 (15.3)	60.7 (16.5)	62.7 (17.5)	65.3 (17.6)	<0.01
Arachidonic acid(C20:4n6), mg/L	155.7 (32.6)	145.8 (29.3)	153.8 (31.1)	162.7 (33.7)	170.3 (36.7)	<0.01
n6/n3 PUFAs ratio, mg/L	6.2 (1.7)	6.2 (1.7)	6.0 (1.7)	6.4 (1.8)	6.6 (1.5)	<0.01

[a]Values represent mean (SD). *Differences in subject characteristics between groups were evaluated using 1-way ANOVA test.

Table S2.1.3. Comparison of subject characteristics according to gestational weight gain (N=2,697)[a]

		Weight gain categories			
	Total Group (2,697)	**Insuficient gestational weight gain (n=546)**	**Sufficient gestational weight gain (n=922)**	**Excessive gestational weight gain (n=1,229)**	***P* Value***
Maternal Characteristics					
Age (years)	29.3 (5.6)	30.7 (5.2)	31.5 (4.4)	30.8 (4.7)	0.02
Height (cm)	169.0 (7.2)	167.8 (6.9)	168.9 (7.3)	169.7 (7.1)	<0.01
Weight (kg)	68.7 (12.1)	65.6 (11.9)	65.6 (10.4)	72.5 (12.6)	<0.01
Body mass index (kg/m^2)	23.2 (3.9)	23.1 (4.3)	22.2 (3.2)	23.9 (4.0)	<0.01
Education, No. higher education (%)	1541 (57.9)	284 (52.8)	583 (64.2)	674 (55.5)	<0.01
Race / ethnicity, No. European (%)	1939 (72.2)	352 (64.7)	680 (74)	907 (74)	<0.01
Parity, No. nulliparous (%)	1635 (60.7)	292 (53.5)	534 (57.9)	809 (65.9)	<0.01
Total energy intake (kcal)	2079 (543)	2066 (567)	2043 (554)	2110 (523)	0.56
Carbohydrates, energy (%)	48.5 (6.2)	48.9 (6.1)	48.1 (6.3)	48.6 (6.2)	0.36
Proteins, energy (%)	15.1 (2.6)	15.2 (2.6)	15.2 (2.5)	14.9 (2.6)	0.49
Fat, energy (%)	36.0 (5.3)	35.5 (5.4)	36.2 (5.3)	36.1 (5.3)	0.38
Folic acid supplement use (yes), No. (%)	1919 (83.6)	360 (77.7)	656 (84.7)	903 (85.2)	<0.01
Smoking during pregnancy (yes), No. (%)	614 (23.8)	80 (15.2)	170 (19.3)	364 (30.9)	<0.01
Alcohol consumption during pregnancy (yes), No. (%)	1598 (61.9)	310 (58.8)	581 (65.9)	707 (60.3)	0.02

[a]Values represent mean (SD), median (95% range), or number of subjects (valid%). *Differences in subject characteristics between groups were evaluated using 1-way ANOVA test.

Table S2.1.4. Associations of maternal gestational weight gain during pregnancy with saturated and monounsaturated fatty acid concentrations unadjusted for prepregnancy body mass index (N = 2,697)[a]

	Difference in saturated fatty acid (SFA) concentrations (95% Confidence Interval)			
	Total SFAs (SD)	**Myristic acid (SD)**	**Palmitic acid (SD)**	**Stearic acid (SD)**
Gestational weight gain[b,c]				
Insufficient gestational weight gain	-0.05 (-0.15, 0.06)	-0.28 (-0.38, -0.18)*	-0.03 (-0.13, 0.07)	-0.05 (-0.15, 0.06)
Sufficient gestational weight gain	*Reference*	*Reference*	*Reference*	*Reference*
Excessive gestational weight gain	0.19 (0.10, 0.27)*	0.11 (0.03, 0.19)*	0.17 (0.09, 0.25)*	0.20 (0.11, 0.28)*
	Difference in monounsaturated fatty acids (MUFAs) concentrations (95% Confidence Interval)			
	Total MUFAs (SD)		**Palmitoleic acid (SD)**	**Oleic acid (SD)**
Gestational weight gain[b,c]				
Insufficient gestational weight gain	-0.04 (-0.14, 0.06)		-0.10 (-0.20, 0)	-0.05 (-0.15, 0.05)
Sufficient gestational weight gain	*Reference*		*Reference*	*Reference*
Excessive gestational weight gain	0.16 (0.08, 0.24)*		0.21 (0.13, 0.30)*	0.15 (0.07, 0.23)*

[a]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of saturated and monounsaturated fatty acid concentrations for insufficient weight gain and excessive weight gain women as compared to women with sufficient weight gain. [b]Models are adjusted for maternal age, educational level, ethnicity, parity, smoking and alcohol consumption, folic acid supplement use and total caloric and fat intake during pregnancy. [c]Models are unadjusted for maternal prepregnancy body mass index. * P-value<0.05

Table S2.1.5. Associations of maternal gestational weight gain during pregnancy with n-3 and n-6 polyunsaturated fatty acid concentrations unadjusted for prepregnancy body mass index (N = 2,697)[a]

	Difference in n-3 Polyunsaturated fatty acids (PUFAs) concentrations (95% Confidence Interval)			
	Total n-3 PUFAs (SD)	**α-Linolenic acid (SD)**	**Eicosapentaenoic acid (SD)**	**Docosahexaenoic acid (SD)**
Gestational weight gain[b,c]				
Insufficient gestational weight gain	-0.07 (-0.18, 0.03)	-0.16 (-0.26, -0.06)*	-0.18 (-0.29, -0.08)*	-0.01 (-0.11, 0.09)
Sufficient gestational weight gain	*Reference*	*Reference*	*Reference*	*Reference*
Excessive gestational weight gain	-0.01 (-0.09, 0.07)	-0.01 (-0.09, 0.08)	-0.01 (-0.09, 0.09)	-0.03 (-0.11, 0.06)
	Difference in n-6 Polyunsaturated fatty acids (PUFAs) concentrations (95% Confidence Interval)			
	Total n-6 PUFAs (SD)	**Linoleic acid (SD)**	**Dihomo-gamma linolenic acid (SD)**	**Arachidonic acid (SD)**
Gestational weight gain[b,c]				
Insufficient gestational weight gain	0.03 (-0.07, 0.13)	0.01 (-0.10, 0.11)	-0.05 (-0.15, 0.05)	0.10 (-0.02, 0.20)
Sufficient gestational weight gain	*Reference*	*Reference*	*Reference*	*Reference*
Excessive gestational weight gain	0.15 (0.07, 0.23)*	0.03 (-0.06, 0.11)	0.36 (0.28, 0.44)*	0.13 (0.04, 0.22)*

[a]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of n-3 and n-6 PUFA concentrations for insufficient weight gain and excessive weight gain women as compared to women with sufficient weight gain. [b]Models are adjusted for maternal age, educational level, ethnicity, parity, smoking and alcohol consumption, folic acid supplement use and total caloric and fat intake during pregnancy. [c]Models are unadjusted for maternal prepregnancy body mass index. * P-value<0.05

Table S2.1.6. Associations of maternal weight during pregnancy with saturated fatty acid concentrations unadjusted for total energy and fat intake (N = 5,636)[a,b]

	Difference in saturated fatty acid (SFA) concentrations (95% Confidence Interval)			
	Total SFAs (SD)	**Myristic acid (SD)**	**Palmitic acid (SD)**	**Stearic acid (SD)**
Body mass index[c]				
Underweight [<20.0 kg/m^2]	-0.17 (-0.24, -0.10)*	-0.03 (-0.10, 0.05)	-0.17 (-0.24, -0.10)*	-0.16 (-0.23, -0.09)*
Normal weight [20.0-24.9 kg/m^2]	*Reference*	*Reference*	*Reference*	*Reference*
Overweight [25.0-29.9 kg/m^2]	0.07 (0.01, 0.14)*	-0.13 (-0.20, -0.07)*	0.07 (0.01, 0.14)*	0.08 (0.02, 0.15)*
Obesity [≥30.0 kg/m^2]	0.08 (-0.01, 0.18)	-0.29 (-0.38, -0.20)*	0.11 (0.01, 0.20)*	0.06 (-0.04, 0.15)
Body mass index (SD)	0.07 (0.04, 0.09)*	-0.09 (-0.11, -0.06)*	0.07 (0.05, 0.10)*	0.06 (0.03, 0.09)*
	Difference in saturated fatty acid (SFA) concentrations (95% Confidence Interval)			
	Total SFAs (SD)	**Myristic acid (SD)**	**Palmitic acid (SD)**	**Stearic acid (SD)**
Gestational weight gain[c]				
Insufficient gestational weight gain	-0.06 (-0.16, 0.05)	-0.26 (-0.36, -0.16)*	-0.04 (-0.14, 0.06)	-0.06 (-0.16, 0.05)
Sufficient gestational weight gain	*Reference*	*Reference*	*Reference*	*Reference*
Excessive gestational weight gain	0.16 (0.08, 0.25)*	0.15 (0.07, 0.23)*	0.15 (0.06, 0.23)*	0.18 (0.09, 0.26)*
Gestational weight gain (SD)[d]	0.09 (0.05, 0.13)*	0.18 (0.14, 0.22)*	0.08 (0.04, 0.11)*	0.10 (0.06, 0.14)*
Gestational weight gain (SD)[e]	0.10 (0.06, 0.14)*	0.17 (0.14, 0.21)*	0.09 (0.05, 0.12)*	0.11 (0.07, 0.15)*

[a]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of saturated fatty acid concentrations for underweight, overweight and obese women as compared to normal weight women, and per SD increase in pre-pregnancy body mass index. Models are adjusted for age, educational level, ethnicity, parity, smoking and alcohol consumption and folic acid supplement use during pregnancy. [b]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of saturated fatty acid concentrations for insufficient weight gain and excessive weight gain women as compared to women with sufficient weight gain, and per SD increase in weight gain. Models are adjusted for age, educational level, ethnicity, parity, smoking and alcohol consumption and folic acid supplement use during pregnancy. Models for weight gain defined according to the IOM criteria are additionally adjusted for pre-pregnancy body mass index. [c]Models are unadjusted for total energy and fat intake. [d]Gestational weight gain SD unadjusted for body mass index. [e]Gestational weight gain SD adjusted for body mass index. * P-value<0.05.

Table S2.1.7. Associations of maternal weight during pregnancy with monounsaturated fatty acid concentrations unadjusted for total energy and fat intake (N =5,636)[a,b]

	Difference in monounsaturated fatty acids (MUFAs) concentrations (95% Confidence Interval)		
	Total MUFAs (SD)	**Palmitoleic acid (SD)**	**Oleic acid (SD)**
Body mass index[c]			
Underweight [<20.0 kg/m^2]	-0.05 (-0.12, 0.02)	-0.12 (-0.19, -0.05)*	-0.02 (-0.09, 0.05)
Normal weight [20.0-24.9 kg/m^2]	*Reference*	*Reference*	*Reference*
Overweight [25.0-29.9 kg/m^2]	-0.02 (-0.09, 0.04)	0.05 (-0.02, 0.12)	-0.03 (-0.10, 0.03)
Obesity [≥30.0 kg/m^2]	-0.04 (-0.13, 0.05)	0.21 (0.11, 0.30)*	-0.09 (-0.18, 0.01)
Body mass index (SD)	-0.01 (-0.03, 0.02)	0.08 (0.05, 0.10)*	-0.02 (-0.05, 0.01)
	Difference in monounsaturated fatty acids (MUFAs) concentrations (95% Confidence Interval)		
	Total MUFAs (SD)	**Palmitoleic acid (SD)**	**Oleic acid (SD)**
Gestational weight gain[c]			
Insufficient gestational weight gain	-0.04 (-0.13, 0.06)	-0.11 (-0.21, -0.01)*	-0.04 (-0.14, 0.06)
Sufficient gestational weight gain	*Reference*	*Reference*	*Reference*
Excessive gestational weight gain	0.16 (0.08, 0.24)*	0.18 (0.10, 0.27)*	0.16 (0.08, 0.24)*
Gestational weight gain (SD)[d]	0.10 (0.06, 0.13)*	0.13 (0.10, 0.17)*	0.10 (0.06, 0.14)*
Gestational weight gain (SD)[e]	0.10 (0.06, 0.14)*	0.15 (0.11, 0.18)*	0.10 (0.06, 0.14)*

[a]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of monounsaturated fatty acid concentrations for underweight, overweight and obese women as compared to normal weight women, and per SD increase in pre-pregnancy body mass index. Models are adjusted for age, educational level, ethnicity, parity, smoking and alcohol consumption and folic acid supplement use during pregnancy. [b]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of monounsaturated fatty acid concentrations for insufficient weight gain, and excessive weight gain women as compared to women with sufficient weight gain, and per SD increase in weight gain. Models are adjusted for age, educational level, ethnicity, parity, smoking and alcohol consumption and folic acid supplement use during pregnancy. [c]Models are unadjusted for total energy and fat intake. Models for weight gain defined according to the IOM criteria are additionally adjusted for pre-pregnancy body mass index. [d]Gestational weight gain SD unadjusted for body mass index. [e]Gestational weight gain SD adjusted for body mass index.* P-value<0.05.

Table S2.1.8. Associations of maternal weight during pregnancy with n-3 polyunsaturated fatty acid concentrations unadjusted for total energy and fat intake (N =5,636)[a,b]

	Difference in n-3 Polyunsaturated fatty acids (PUFAs) concentrations (95% Confidence Interval)			
	Total n-3 PUFAs (SD)	**α-Linolenic a cid (SD)**	**Eicosapentaenoic acid (SD)**	**Docosahexaenoic acid (SD)**
Body mass index[c]				
Underweight [<20.0 kg/m^2]	-0.11 (-0.18, -0.05)*	0.06 (-0.01, 0.13)	-0.03 (-0.10, 0.04)	-0.13 (-0.20, -0.06)*
Normal weight [20.0-24.9 kg/m^2]	*Reference*	*Reference*	*Reference*	*Reference*
Overweight [25.0-29.9 kg/m^2]	-0.04 (-0.11, 0.02)	-0.18 (-0.24, -0.11)*	-0.05 (-0.11, 0.02)	-0.01 (-0.07, 0.06)
Obesity [≥30.0 kg/m^2]	-0.10 (-0.19, -0.02)*	-0.34 (-0.43, -0.25)*	-0.09 (-0.18, 0.01)	-0.04 (-0.13, 0.05)
Body mass index (SD)	-0.02 (-0.04, 0.01)	-0.13 (-0.15, -0.10)*	-0.02 (-0.05, 0.01)	0.01 (-0.02, 0.03)
	Difference in n-3 Polyunsaturated fatty acids (PUFAs) concentrations (95% Confidence Interval)			
	Total n-3 PUFAs (SD)	**α-Linolenic acid (SD)**	**Eicosapentaenoic acid (SD)**	**Docosahexaenoic acid (SD)**
Gestational weight gain[c]				
Insufficient gestational weight gain	-0.07 (-0.17, 0.04)	-0.14 (-0.24, -0.04)*	-0.18 (-0.28, -0.07)*	-0.01 (-0.11, 0.10)
Sufficient gestational weight gain	*Reference*	*Reference*	*Reference*	*Reference*
Excessive gestational weight gain	0.01 (-0.08, 0.09)	0.05 (-0.03, 0.14)	0.02 (-0.07, 0.11)	-0.02 (-0.11, 0.06)
Gestational weight gain (SD)[d]	0.03 (-0.01, 0.06)	0.09 (0.05, 0.13)*	0.08 (0.04, 0.12)*	-0.02 (-0.06, 0.02)
Gestational weight gain (SD)[e]	0.02 (-0.02, 0.06)	0.07 (0.04, 0.11)*	0.08 (0.04, 0.12)*	-0.02 (-0.06, 0.02)

[a]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of n-3 PUFA concentrations for underweight, overweight and obese women as compared to normal weight women, and per SD increase in pre-pregnancy body mass index. Models are adjusted for, age, educational level, ethnicity, parity, smoking and alcohol consumption and folic acid supplement use during pregnancy.

[b]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of n-3 PUFA concentrations for insufficient weight gain, and excessive weight gain women as compared to women with sufficient weight gain, and per SD increase in weight gain. Models are adjusted for age, educational level, ethnicity, parity, smoking and alcohol consumption and folic acid supplement use during pregnancy. [c]Models are unadjusted for total energy and fat intake. Models for weight gain defined according to the IOM criteria are additionally adjusted for pre-pregnancy body mass index. [d]Gestational weight gain SD unadjusted for body mass index. [e]Gestational weight gain SD adjusted for body mass index.* P-value<0.05.

Table S2.1.9. Associations of maternal weight during pregnancy with n-6 polyunsaturated fatty acid concentrations unadjusted for total energy and fat intake (N = 5,636)[a,b]

	Difference in n-6 Polyunsaturated fatty acids (PUFAs) concentrations (95% Confidence Interval)			
	Total n-6 PUFAs (SD)	**Linoleic acid (SD)**	**Dihomo-γ-linolenic acid (SD)**	**Arachidonic acid (SD)**
Body mass index[c]				
Underweight [<20.0 kg/m^2]	-0.16 (-0.24, -0.09)*	-0.03 (-0.10, 0.04)	-0.27 (-0.34, -0.20)*	-0.23 (-0.30, -0.16)*
Normal weight [20.0-24.9 kg/m^2]	*Reference*	*Reference*	*Reference*	*Reference*
Overweight [25.0-29.9 kg/m^2]	0.09 (0.02, 0.16)*	-0.02 (-0.09, 0.05)	0.16 (0.09, 0.23)*	0.23 (0.16, 0.30)*
Obesity [≥30.0 kg/m^2]	0.05 (-0.05, 0.14)	-0.23 (-0.32, -0.13)*	0.34 (0.24, 0.43)*	0.44 (0.34, 0.53)*
Body mass index (SD)	0.06 (0.03, 0.09)*	-0.05 (-0.08, -0.02)*	0.16 (0.13, 0.18)*	0.19 (0.16, 0.22)*
	Difference in n-6 Polyunsaturated fatty acids (PUFAs) concentrations (95% Confidence Interval)			
	Total n-6 PUFAs (SD)	**Linoleic acid (SD)**	**Dihomo-γ-linolenic acid (SD)**	**Arachidonic acid (SD)**
Gestational weight gain[c]				
Insufficient gestational weight gain	0.02 (-0.09, 0.12)	0.01 (-0.09, 0.11)	-0.08 (-0.18, 0.02)	0.07 (-0.03, 0.17)
Sufficient gestational weight gain	*Reference*	*Reference*	*Reference*	*Reference*
Excessive gestational weight gain	0.13 (0.04, 0.21)*	0.05 (-0.04, 0.14)	0.29 (0.21, 0.38)*	0.05 (-0.03, 0.13)
Gestational weight gain (SD)[d]	0.04 (0.01, 0.08)*	0.02 (-0.02, 0.06)	0.15 (0.11, 0.19)*	-0.03 (-0.06, 0.01)
Gestational weight gain (SD)[e]	0.05 (0.02, 0.09)*	0.01 (-0.02, 0.05)	0.18 (0.14, 0.22)*	-0.00 (-0.04, 0.04)

[a]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of n-6 PUFA concentrations for underweight, overweight and obese women as compared to normal weight women, and per SD increase in pre-pregnancy body mass index. Models are adjusted for, age, educational level, ethnicity, parity, smoking and alcohol consumption and folic acid supplement use during pregnancy. [b]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of n-6 PUFA concentrations for insufficient weight gain, and excessive weight gain women as compared to women with sufficient weight gain, and per SD increase in weight gain. Models are adjusted for maternal body mass index, age, educational level, ethnicity, parity, smoking and alcohol consumption and folic acid supplement use during pregnancy. [c]Models are unadjusted for total energy and fat intake. Models for weight defined according to the IOM criteria are additionally adjusted for pre-pregnancy body mass index. [d]Gestational weight gain SD unadjusted for body mass index. [e]Gestational weight gain SD adjusted for body mass index.* P-value<0.05.

Table S2.1.10. Associations of maternal weight during pregnancy with saturated fatty acid concentrations unadjusted for maternal characteristics (N = 5,636)[a,b]

	Difference in saturated fatty acid (SFA) concentrations (95% Confidence Interval)			
	Total SFAs (SD)	**Myristic acid (SD)**	**Palmitic acid (SD)**	**Stearic acid (SD)**
Body mass index[c]				
Underweight [<20.0 kg/m^2]	-0.20 (-0.28, -0.13)*	-0.05 (-0.12, 0.02)	-0.20 (-0.28, -0.13)*	-0.18 (-0.25, -0.10)*
Normal weight [20.0-24.9 kg/m^2]	*Reference*	*Reference*	*Reference*	*Reference*
Overweight [25.0-29.9 kg/m^2]	0.04 (-0.03, 0.11)*	-0.19 (-0.26, -0.12)*	0.03 (-0.04, 0.10)	0.09 (0.02, 0.16)*
Obesity [≥30.0 kg/m^2]	0.03 (-0.06, 0.12)*	-0.39 (-0.48, -0.29)*	0.03 (-0.06, 0.13)	0.07 (-0.03, 0.16)
Body mass index (SD)	0.06 (0.03, 0.08)*	-0.11 (-0.14, -0.09)*	0.06 (0.03, 0.08)*	0.07 (0.04. 0.09)*
	Difference in saturated fatty acid (SFA) concentrations (95% Confidence Interval)			
	Total SFAs (SD)	**Myristic acid (SD)**	**Palmitic acid (SD)**	**Stearic acid (SD)**
Gestational weight gain[c]				
Insufficient gestational weight gain	-0.08 (-0.18, 0.02)	-0.30 (-0.40, -0.20)*	-0.07 (-0.17, 0.03)	-0.06 (-0.16, 0.04)
Sufficient gestational weight gain	*Reference*	*Reference*	*Reference*	*Reference*
Excessive gestational weight gain	0.17 (0.09, 0.25)*	0.16 (0.08, 0.24)*	0.15 (0.07, 0.23)*	0.19 (0.10, 0.27)*
Gestational weight gain (SD)[d]	0.09 (0.05, 0.13)*	0.18 (0.14, 0.22)*	0.08 (0.04, 0.11)*	0.10 (0.06, 0.14)*
Gestational weight gain (SD)[e]	0.10 (0.06, 0.14)*	0.17 (0.13, 0.21)*	0.09 (0.05, 0.12)*	0.11 (0.07, 0.15)*

[a]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of saturated fatty acid concentrations for underweight, overweight and obese women as compared to normal weight women, and per SD increase in pre-pregnancy body mass index. [b]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of saturated fatty acid concentrations for insufficient weight gain and excessive weight gain women as compared to women with sufficient weight gain, and per SD increase in weight gain. [c]Models are unadjusted for potential confounders. Models for weight gain defined according to the IOM criteria are additionally adjusted for pre-pregnancy body mass index. [d]Gestational weight gain SD unadjusted for body mass index. [e]Gestational weight gain SD adjusted for body mass index. * P-value<0.05.

Table S2.1.11. Associations of maternal weight during pregnancy with monounsaturated fatty acid concentrations unadjusted for maternal characteristics (N = 5,636)[a,b]

	Difference in monounsaturated fatty acids (MUFAs) concentrations (95% Confidence Interval)		
	Total MUFAs (SD)	**Palmitoleic acid (SD)**	**Oleic acid (SD)**
Body mass index[c]			
Underweight [<20.0 kg/m^2]	-0.06 (-0.14, 0.01)	-0.12 (-0.19, -0.05)*	-0.05 (-0.12, 0.03)
Normal weight [20.0-24.9 kg/m^2]	*Reference*	*Reference*	*Reference*
Overweight [25.0-29.9 kg/m^2]	-0.14 (-0.20, -0.07)*	-0.02 (-0.09, 0.05)	-0.15 (-0.22, -0.08)*
Obesity [≥30.0 kg/m^2]	-0.22 (-0.31, -0.12)*	0.11 (0.01, 0.20)*	-0.26 (-0.36, -0.17)*
Body mass index (SD)	-0.06 (-0.08, -0.03)*	0.05 (0.02, 0.07)*	-0.07 (-0.10, -0.05)*

	Difference in monounsaturated fatty acids (MUFAs) concentrations (95% Confidence Interval)		
	Total MUFAs (SD)	**Palmitoleic acid (SD)**	**Oleic acid (SD)**
Gestational weight gain[c]			
Insufficient gestational weight gain	-0.10 (-0.20, 0)	-0.15 (-0.25, -0.05)*	-0.10 (-0.20, 0)
Sufficient gestational weight gain	*Reference*	*Reference*	*Reference*
Excessive gestational weight gain	0.17 (0.09, 0.26)*	0.22 (0.14, 0.31)*	0.17 (0.09, 0.25)*
Gestational weight gain (SD)[d]	0.10 (0.06, 0.13)*	0.13 (0.10, 0.17)*	0.10 (0.06, 0.14)*
Gestational weight gain (SD)[e]	0.10 (0.06, 0.14)*	0.15 (0.11, 0.19)*	0.10 (0.06, 0.14)*

[a]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of monounsaturated fatty acid concentrations for underweight, overweight and obese women as compared to normal weight women, and per SD increase in prepregnancy body mass index. [b]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of monounsaturated fatty acid concentrations for insufficient weight gain and excessive weight gain women as compared to women with sufficient weight gain, and per SD increase in weight gain. [c]Models are unadjusted for potential confounders. Models for weight gain defined according to the IOM criteria are additionally adjusted for prepregnancy body mass index. [d]Gestational weight gain SD unadjusted for body mass index. [e]Gestational weight gain SD adjusted for body mass index. * P-value<0.05.

Table S2.1.12. Associations of maternal weight during pregnancy with n-3 polyunsaturated fatty acid concentrations unadjusted for maternal characteristics (N = 5,636)[a]

	Difference in n-3 Polyunsaturated fatty acids (PUFAs) concentrations (95% Confidence Interval)			
	Total n-3 PUFAs (SD)	**α-Linolenic acid (SD)**	**Eicosapentaenoic acid (SD)**	**Docosahexaenoic acid (SD)**
Body mass index[c]				
Underweight [<20.0 kg/m^2]	-0.16 (-0.24, -0.09)*	0.04 (-0.03, 0.12)	-0.07 (-0.15, 0.01)	-0.18 (-0.26, -0.11)*
Normal weight [20.0-24.9 kg/m^2]	*Reference*	*Reference*	*Reference*	*Reference*
Overweight [25.0-29.9 kg/m^2]	-0.16 (-0.23, -0.09)*	-0.25 (-0.32, -0.18)*	-0.17 (-0.23, -0.10)*	-0.13 (-0.19, -0.06)*
Obesity [≥30.0 kg/m^2]	-0.32 (-0.41, -0.22)*	-0.44 (-0.54, -0.35)*	-0.30 (-0.40, -0.21)*	-0.26 (-0.36, -0.17)*
Body mass index (SD)	-0.07 (-0.10, -0.05)*	-0.16 (-0.18, -0.13)*	-0.08 (-0.11, -0.06)*	-0.05 (-0.08, -0.02)*
	Difference in n-3 Polyunsaturated fatty acids (PUFAs) concentrations (95% Confidence Interval)			
	Total n-3 PUFAs (SD)	**α-Linolenic acid (SD)**	**Eicosapentaenoic acid (SD)**	**Docosahexaenoic acid (SD)**
Gestational weight gain[c]				
Insufficient gestational weight gain	-0.15 (-0.25, -0.04)*	-0.18 (-0.28, -0.07)*	-0.25 (-0.36, -0.14)*	-0.08 (-0.19, 0.03)
Sufficient gestational weight gain	*Reference*	*Reference*	*Reference*	*Reference*
Excessive gestational weight gain	-0.01 (-0.10, 0.08)	0.07 (-0.01, 0.15)	0.01 (-0.08, 0.10)	-0.04 (-0.13, 0.05)
Gestational weight gain (SD)[d]	0.03 (-0.01, 0.06)	0.09 (0.05, 0.13)*	0.08 (0.04, 0.12)*	-0.02 (-0.06, 0.02)
Gestational weight gain (SD)[e]	0.02 (-0.02, 0.06)	0.07 (0.04, 0.11)*	0.08 (0.04, 0.12)*	-0.02 (-0.06, 0.02)

[a]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of n-3 PUFA concentrations for underweight, overweight and obese women as compared to normal weight women, and per SD increase in pre-pregnancy body mass index. [b]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of n-3 PUFA concentrations for insufficient weight gain, and excessive weight gain women as compared to women with sufficient weight gain, and per SD increase in weight gain. [c]Models are unadjusted for potential confounders. Models for weight gain defined according to the IOM criteria are additionally adjusted for pre-pregnancy body mass index. [c]Gestational weight gain SD unadjusted for body mass index. [d]Gestational weight gain SD adjusted for body mass index.* P-value<0.05.

Table S2.1.13. Associations of maternal weight during pregnancy with n-6 polyunsaturated fatty acid concentrations unadjusted for maternal characteristics (N = 5,636)[a,b]

	Difference in n-6 Polyunsaturated fatty acids (PUFAs) concentrations (95% Confidence Interval)			
	Total n-6 PUFAs (SD)	**Linoleic acid (SD)**	**Dihomo-γ-linolenic acid (SD)**	**Arachidonic acid (SD)**
Body mass index[c]				
Underweight [<20.0 kg/m^2]	-0.18 (-0.26, -0.11)*	-0.04 (-0.12, 0.03)	-0.28 (-0.36, -0.21)*	-0.25 (-0.32, -0.17)*
Normal weight [20.0-24.9 kg/m^2]	*Reference*	*Reference*	*Reference*	*Reference*
Overweight [25.0-29.9 kg/m^2]	0.16 (0.10, 0.23)*	0.05 (-0.02, 0.11)	0.12 (0.05, 0.19)*	0.28 (0.21, 0.34)*
Obesity [≥30.0 kg/m^2]	0.15 (0.06, 0.25)*	-0.13 (-0.22, -0.04)*	0.28 (0.18, 0.37)*	0.51 (0.42, 0.60)*
Body mass index (SD)	0.10 (0.08, 0.13)*	-0.01 (-0.04, 0.01)	0.14 (0.12, 0.17)*	0.22 (0.19, 0.24)*
	Difference in n-6 Polyunsaturated fatty acids (PUFAs) concentrations (95% Confidence Interval)			
	Total n-6 PUFAs (SD)	**Linoleic acid (SD)**	**Dihomo-γ-linolenic acid (SD)**	**Arachidonic acid (SD)**
Gestational weight gain[c]				
Insufficient gestational weight gain	0.05 (-0.06, 0.15)	0.05 (-0.06, 0.15)	-0.10 (-0.19, 0.01)	0.08 (-0.02, 0.18)
Sufficient gestational weight gain	*Reference*	*Reference*	*Reference*	*Reference*
Excessive gestational weight gain	0.13 (0.04, 0.21)*	0.05 (-0.04, 0.13)	0.31 (0.23, 0.39)*	0.06 (-0.03, 0.14)
Gestational weight gain (SD)[d]	0.04 (0.01, 0.08)*	0.02 (-0.02, 0.06)	0.15 (0.11, 0.19)*	-0.03 (-0.06, 0.01)
Gestational weight gain (SD)[e]	0.05 (0.02, 0.09)*	0.01 (-0.02, 0.05)	0.18 (0.14, 0.22)*	-0.00 (-0.04, 0.04)

[a]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of n-6 PUFA concentrations for underweight, overweight and obese women as compared to normal weight women, and per SD increase in pre-pregnancy body mass index. [b]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SD of n-6 PUFA concentrations for insufficient weight gain, and excessive weight gain women as compared to women with sufficient weight gain, and per SD increase in weight gain. [c]Models are unadjusted for potential confounders. Models for weight defined according to the IOM criteria are additionally adjusted for pre-pregnancy body mass index. [c]Gestational weight gain SD unadjusted for body mass index. [d]Gestational weight gain SD adjusted for body mass index.* P-value<0.05.

Chapter 2.2

Fatty acids and hemodynamic adaptations during pregnancy

Aleksandra Jelena Vidakovic, Vincent W.V. Jaddoe, Hans Demmelmair, Berthold Koletzko, Henning Tiemeier, Eric A.P. Steegers, Romy Gaillard

Submitted

Abstract

Background: An adverse fatty acids profile is associated with cardiovascular disease in adults. We examined whether fatty acid concentrations during pregnancy also affects blood pressure development, placental vascular resistance and the risk of gestational hypertensive disorders.

Methods and Results: This study was embedded in a population-based prospective cohort study among 6,823 pregnant women. We measured saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), n-3 polyunsaturated fatty acid (n-3 PUFA) and n-6 polyunsaturated fatty acid (n-6 PUFA) concentrations at a median gestational age of 20.5 (95% range: 16.5, 24.9) weeks. We repeatedly measured blood pressure in each trimester of pregnancy. Umbilical and uterine artery vascular resistances were assessed in the second and third trimester. Information about doctor diagnosed gestational hypertensive disorders was obtained from medical records. We observed that higher maternal SFA concentrations, but not MUFA concentrations, were associated with higher blood pressure from first trimester onwards (*P* values<0.05). Higher total maternal n-3 PUFA and n-6 PUFA concentrations, especially docosahexaenoic acid (DHA) and linoleic acid (LA), were associated with a lower blood pressure throughout pregnancy (*P* values<0.05). Maternal fatty acid concentrations were not consistently associated with placental vascular resistance indices. Only higher maternal SFA concentrations were associated with the risk of gestational hypertensive disorders (Odds Ratio (OR) 1.23 (95% CI: 1.09, 1.37) per SD increase in total SFAs).

Conclusions: An adverse maternal fatty acids profile, characterized by higher concentrations of SFA and lower concentrations of PUFA, is associated with higher maternal blood pressure levels throughout pregnancy, but not with placental vascular resistance indices. Higher maternal SFA, but not other fatty acids, were associated with an increased risk of gestational hypertensive disorders. Further studies are needed to identify through which pathways different fatty acids affect maternal hemodynamic adaptations during pregnancy.

Introduction

An adverse fatty acids profile may lead to increased risks of cardiovascular disease.[1-3] Results from observational studies and randomized controlled trials suggest that higher saturated fatty acids (SFA) concentrations adversely affect cholesterol metabolism and are associated with increased risks of hypertension and coronary heart disease.[1,4] In contrast, higher n-3 polyunsaturated fatty acids (PUFA) concentrations, and linoleic acid (LA), a n-6 PUFA, seem to have beneficial effects on blood pressure levels and endothelial function.[5-7] For the current study, we hypothesized that adverse fatty acid profile, characterized by higher concentrations of SFA and lower concentrations of PUFA also lead to hemodynamic maladaptations during pregnancy and increased risks of gestational hypertensive disorders. N-3 PUFAs act as anti-oxidative and anti-inflammatory agents and may stimulate placental angiogenesis, which is beneficial for adequate placental development.[8,9] Conversely, SFA and unsaturated fatty acids might increase placental inflammatory cytokines, decrease uterine volume blood flow and adversely affect placental efficiency resulting in placental dysfunction.[10,11] Abnormal placental development leads to pregnancy-related hemodynamic maladaptions and gestational hypertensive disorders.[12] Thus far, results from observational studies suggest that higher n-3 PUFA concentrations during pregnancy are associated with a lower risk of pre-eclampsia, whereas higher SFA and n-6 PUFA concentrations are associated with increased risks of pre-eclampsia.[13,14] However, results are not consistent, which may be due to differences in study populations and adjustment for confounding factors.[13-16] In addition, not much is known about the direct associations of different fatty acid concentrations with blood pressure development during pregnancy and placental vascular function.

Therefore, in a population based prospective cohort study from early pregnancy onwards among 6,823 pregnant women, we assessed the associations of maternal plasma fatty acid concentrations with longitudinal blood pressure development, placental vascular function throughout pregnancy and the risks of gestational hypertensive disorders.

Methods

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands.[17,18] The study has been approved by medical Ethical Committee of Erasmus Medical Center,

Rotterdam. Written consent was obtained from all participating women.[19] Response rate at birth was 61%. In total, 8,879 women were enrolled during their pregnancy, of whom 6,999 had information on mid-pregnancy fatty acid concentrations available. We excluded pregnancies not leading to singleton live-born children and women with preexisting hypertension or without information on blood pressure or placental vascular function available, leading to a population for analysis of 6,823 pregnant women (**Supplemental Figure S2.2.1**).

Fatty acids measurements

Venous samples were drawn at a median gestational age of 20.5 weeks (95% range: 16.5, 24.9). To analyze fatty acid concentrations, EDTA plasma samples were selected and transported to the Division of Metabolic Diseases and Nutritional Medicine, Dr. von Hauner Children's Hospital, University of Munich Medical Center. After being thawed, the analysis of plasma glycerophospholipid fatty acids was performed by a sensitive and precise high throughput method. This method is suitable for applications in large epidemiological studies.[20] Based on findings from previous studies, we selected fatty acids for our analyses, which have been associated with the risk of cardiovascular disease in adults and adverse pregnancy outcomes.[2,21-25] Selected saturated fatty acids included total SFA concentrations, myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0). Monounsaturated fatty acids included total MUFA concentrations, palmitoleic acid (C16:1n7) and oleic acid (C18:1n9). Polyunsaturated fatty acid included total n-3 PUFA concentrations, α-linolenic acid (ALA, C18:3n3), eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3), and total n-6 PUFA concentrations, linoleic acid (LA, C18:2n6), γ-linoleic acid (GLA, C18:3n6) and arachidonic acid (AA, C20:4n6). The ratio of total n-6/n-3 PUFA was calculated. The fatty acid concentrations were expressed as proportion of total fatty acids present in the chromatogram (weight percentage, wt%) to express the relative importance of a fatty acids set against the total fatty acids concentrations.[26]

Blood pressure measurements

We measured blood pressure with the validated Omron 907® automated digital oscillometric sphygmanometer (OMRON Healthcare Europe B.V. Hoofddorp, the Netherlands).[27] All pregnant women were seated in upright position with back support, and were asked to relax for 5 min. A cuff was placed around the non-dominant upper arm, which was supported at the level of the heart, with the bladder midline over the brachial artery pulsation. In case of an upper arm exceeding 33 cm, a larger cuff (32–42 cm) was used. The mean value of 2 blood pressure readings over a 60 s

interval was documented for each participant. In total, we measured blood pressure in 5,246 women in first trimester (median 13.2 weeks of gestation, 95% range 9.5, 17.4), in 6,790 women in second trimester (median 20.4 weeks of gestation, range 18.5-23.5), and in 6,469 women in third trimester (median 30.2 weeks of gestation, 95% range 28.6-32.8). For the analysis, 18,505 blood pressure measurements were available.

Placental vascular resistance indices

We measured placental vascular resistance with recorded flow velocity waveforms from the umbilical and uterine arteries in second and third trimester, as described previously.[12] Umbilical and uterine artery vascular resistance indices are parameters of the feto-placental circulation and utero-placental circulation, respectively.[28] A raised uterine artery resistance index and umbilical artery pulsatility index indicate increased placental resistance.[28] We measured umbilical artery pulsatility index in a free-floating loop of the umbilical cord and uterine artery resistance index near the crossover with the external iliac artery. For each measurement, three consecutive uniform waveforms were recorded by pulsed Doppler ultrasound, during fetal apnea and without fetal movement. We used the mean of three measurements for further analysis.[29]

Gestational hypertensive disorders

We obtained information on pregnancy complications from medical records. Women suspected of pregnancy complications based on these records were crosschecked with the original hospital charts, as described elsewhere.[30] Briefly, the following criteria were used to identify women with gestational hypertension: development of systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg after 20 weeks of gestation in previously normotensive women. These criteria plus the presence of proteinuria (defined as two or more dipstick readings of 2+ or greater, one catheter sample reading of 1+ or greater, or a 24 h urine collection containing at least 300 mg of protein) were used to identify women with preeclampsia.[31] Information on pregnancy complications was available for 6,639 women.

Covariates

Gestational age was established by fetal ultrasonography during the first ultrasound visit.[17] Information on age was assessed at intake. Maternal weight and height were assessed at enrollment, and body mass index was calculated. We collected information about parity, ethnicity, education level, smoking, alcohol consumption, and folic

acid supplementation use by questionnaires. First trimester total energy intake was obtained by a food frequency questionnaire at enrolment.

Statistical analysis

First, we explored the associations of maternal fatty acid concentrations with repeatedly measured systolic and diastolic blood pressure using unbalanced repeated measurement regression models. These models take the correlation between repeated measurements of the same subject into account, and allow for incomplete outcome data.[32] They are described in detail in the Supplementary Material. Second, we examined the associations of maternal fatty acid concentrations with systolic and diastolic blood pressure in each trimester of pregnancy, placental vascular function in the second and third trimester of pregnancy and with presence of third-trimester notching using regular multivariate linear and logistic regression models. Finally, we assessed the associations of maternal fatty acid concentrations with the risk of gestational hypertensive disorders using multivariate logistic regression models. The effect estimates for these associations are shown per standard deviation change in maternal fatty acid concentrations to enable comparison of effect estimates. All models were adjusted for gestational age at enrollment and at blood pressure or placental vascular resistance measurement, age, parity, ethnicity, educational level, prepregnancy body mass index, smoking, alcohol consumption, folic acid supplementation use, and total calorie intake during pregnancy. These covariates were selected based on their associations with the outcomes of interest based on previous studies or a change in effect estimate of >10%. Missing data of covariates were imputed using multiple imputations. Five imputed dataset were created and analysed together. The repeated measurement analysis was performed using the Statistical Analysis System version 9.2 (SAS, Institute Inc. Gary NC, USA), including the Proc Mixed module for unbalanced repeated measurements. All other analyses were performed using the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Subject characteristics

Characteristics of the participants are shown in **Table 2.2.1.** Second trimester maternal total SFA, MUFA, n-3 and n-6 PUFA concentrations were 695.2 mg/L (102.2), 205.5 mg/L (40.1), 101.9 mg/L (27.2) and 603.4 mg/L (89.1), respectively. Of all women, 240 women developed gestational hypertension and 146 women developed

preeclampsia. Non-response analysis showed that those women without follow-up measurements had a higher body mass index and higher concentrations of SFA, MUFA, and n-6 PUFA (**Supplemental Table S2.2.1**). **Supplemental Table S2.2.2** shows the correlation between all fatty acid concentrations.

Table 2.2.1. Maternal characteristics (n=6,823)[1]

Maternal characteristics	Value
Age, mean (SD), years	29.7 (5.2)
Height, mean (SD), (cm)	167.3 (7.4)
Prepregnancy body mass index, (kg/m2), mean (SD)	23.6 (4.3)
Gestational age at fatty acid measures, (weeks), median (95% range)	20.5 (16.5, 24.9)
Education, No. (%)	
Primary	695 (11.1)
Secondary	2906 (46.3)
Higher	2672 (23.5)
Ethnicity, No. (%)	
Dutch or European	3254 (50.0)
Non – European	3255 (50.0)
Parity, No. (%)	
Nulliparous	3790 (56.1)
Multiparous	2968 (43.9)
Total calorie intake, kcal, mean (SD)	2043 (568)
Folic acid supplement use, No. (%)	
Yes	3654 (71.6)
No	1450 (28.4)
Smoking, N. (%)	
Yes	1125 (18.7)
No	4889 (81.3)
Saturated fatty acids (SFAs)	
Total SFAs	695.2 (102.2)
Myristic acid (C14:0)	10.5 (3.6)
Palmitic acid (C16:0)	493.9 (76.6)
Stearic acid (C18:0)	185.0 (27.8)
Monounsaturated fatty acids (MUFAs)	
Total MUFAs	205.5 (40.1)
Palmitoleic acid (C16:1n7)	12.0 (5.6)
Oleic acid (C18:1n9)	165.7 (32.9)
n-3 Polyunsaturated fatty acids (PUFAs)	
Total n-3 PUFAs	101.9 (27.2)
α-Linolenic acid (C18:3n3)	5.0 (1.9)
Eicosapentaenoic acid (C20:5n3)	8..4 (5.3)
Docosahexaenoic acid (C22:6n3)	76.5 (20.3)
n-6 Polyunsaturated fatty acids (PUFAs)	
Total n-6 PUFAs	603.4 (89.1)
Linoleic acid (C18:2n6)	361.5 (62.9)

Table 1 Maternal characteristics (n=6,823)[1] (continued)

Maternal characteristics	Value
Y-Linoleic acid (C18:3n6)	1.5 (0.68)
Arachidonic acid (C20:4n6)	156.5 (32.6)
n-6/n-3 PUFAs ratio	6.3 (1.8)
Blood pressure measurements	
First trimester	
Systolic blood pressure, mean (SD), mmHg	115.4 (12.0)
Diastolic blood pressure, mean (SD), mmHg	68.1 (9.3)
Second trimester	
Systolic blood pressure, mean (SD), mmHg	116.4 (11.9)
Diastolic blood pressure, mean (SD), mmHg	66.9 (9.2)
Third trimester	
Systolic blood pressure, mean (SD), mmHg	118.1 (12.0)
Diastolic blood pressure, mean (SD), mmHg	68.9 (9.2)
Placental vascular function	
Second trimester placental resistance indices	
Umbilical artery pulsatility index, mean (SD)	1.2 (0.19)
Uterine artery resistance index, mean (SD)	0.54 (0.09)
Third trimester placental resistance indices	
Umbilical artery pulsatility index, mean (SD)	0.98 (0.17)
Uterine artery resistance index, mean (SD)	0.48 (0.08)
Gestational hypertensive disorders	
Gestational hypertension, No. (%), Yes	240 (3.7)
Pre-eclampsia, No. (%), Yes	146 (2.3)

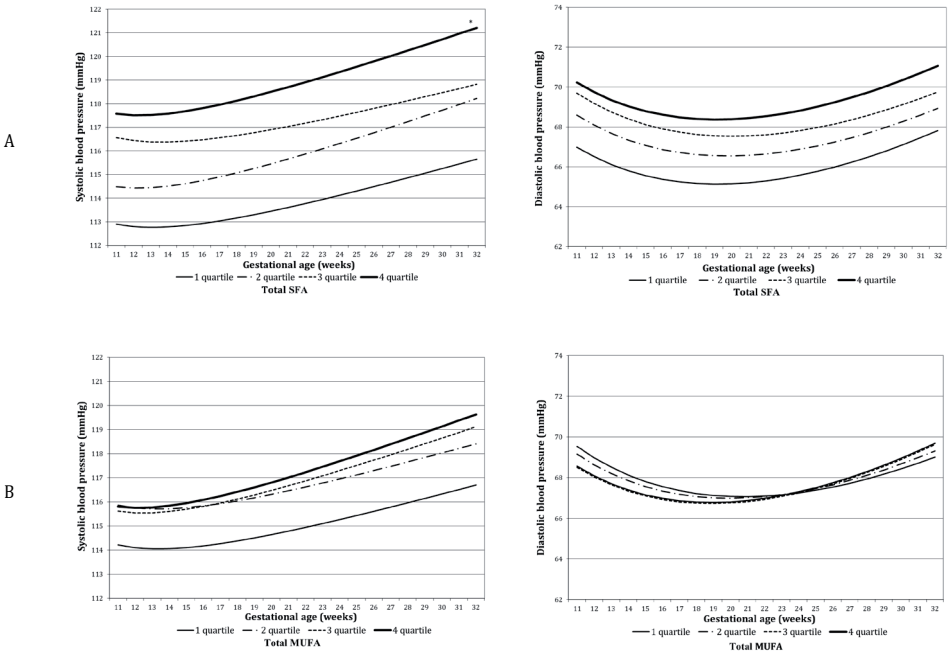
[1]Values are means (standard deviations) or medians (95% range) or observed numbers (valid percentages). Valid percentages represent the percentage of only non-missing cases in each category of categorical variables.

Maternal fatty acids and blood pressure development during pregnancy

Figure 2.2.1 (A and B) shows the systolic and diastolic blood pressure development during pregnancy for women in different total SFA and MUFA quartiles. Systolic blood pressure was higher from first trimester onwards in women in the highest quartile of total SFA (*P* value<0.05).

The steepest increase in systolic blood pressure was also observed for women in the highest total SFA quartile (*P* value<0.05). Diastolic blood pressure showed a mid-pregnancy dip for women in all total SFA quartiles, and was highest for women in the highest total SFA quartile throughout pregnancy (*P* value<0.05). As compared to women in the lowest total MUFA quartile, women in higher total MUFA quartiles had a higher systolic blood pressure throughout pregnancy. No differences in diastolic blood pressure were present for different MUFA quartiles.

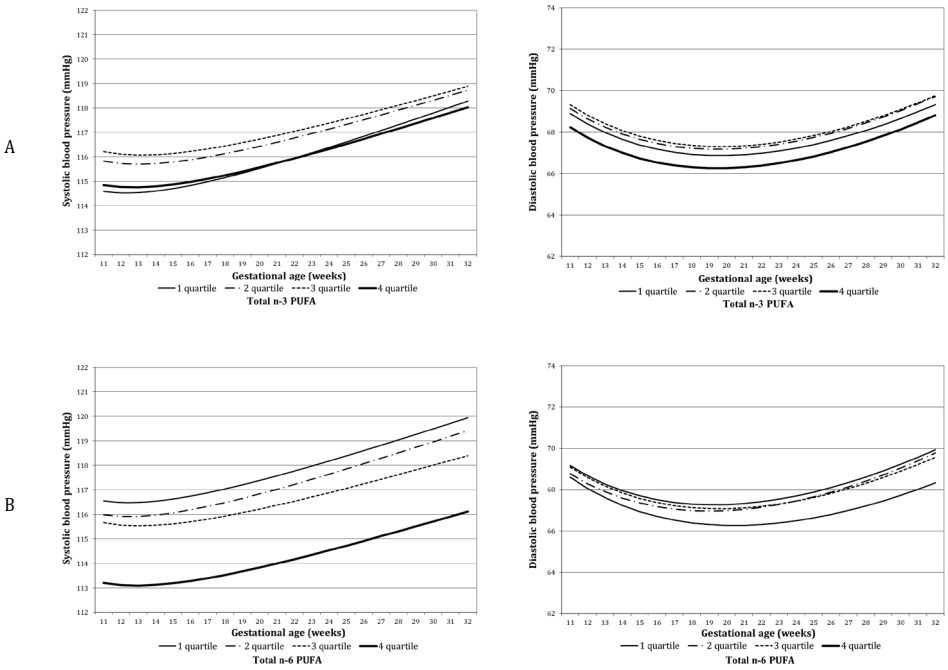
Figure 2.2.1 Maternal SFA and MUFA concentrations with blood pressure in different trimesters



Change patterns in systolic and diastolic blood pressure in mmHg for women with SFA and MUFA concentrations in the second, third and fourth quartiles as compared with women in the first quartile based on repeated measurements analysis. *P < 0.05. *P*-value reflects the significance level of β_4, which reflects the difference in change in blood pressure per week per fourth quartile of SFA concentrations, when compared with first quartile of SFA concentrations.

Figure 2.2.2 (A and B) shows the systolic and diastolic blood pressure development during pregnancy for women in different total n-3 and n-6 PUFA quartiles. Women in the highest total n-3 PUFA quartile had a lower diastolic blood pressure from first onwards, but not systolic blood pressure, as compared to women in the lower quartiles of total n-3PUFA (*P* value<0.05). Women in the highest total n-6 PUFA quartile had a lower systolic and diastolic blood pressure throughout pregnancy, as compared to women in the lower total n-6 PUFA quartiles (*P* value<0.05).

Figure 2.2.2. Maternal n-3 and n-6 PUFA concentrations with blood pressure in different trimesters



Change patterns in systolic and diastolic blood pressure in mmHg for women with n-3 and n-6 PUFA concentrations in the second, third and fourth quartiles as compared with women in the first quartile based on repeated measurements analysis.

Table 2.2.2 shows the associations of different fatty acid concentrations with blood pressure in each trimester of pregnancy from regular linear regression models. Higher total SFA concentrations were associated with a higher systolic and diastolic blood pressure from first trimester onwards (all *P* values<0.05). Among the individual SFA concentrations, palmitic acid were associated with a higher systolic and diastolic blood pressure from first trimester onwards (all *P* values<0.05). Of the MUFAs, only palmitoleic acid concentrations were associated with a higher systolic and diastolic blood pressure from first trimester onwards (all *P* values<0.05). Higher n-3 PUFA concentrations and especially DHA concentrations were associated with lower second and third trimester systolic and diastolic blood pressure (all *P* values<0.05). Higher total n-6 PUFAs and especially LA concentrations, were also associated with lower systolic and diastolic blood pressure from first trimester onwards, whereas higher concentrations of AA were associated with a higher second and third trimester systolic and diastolic blood pressure (all *P* values<0.05). Higher n-6/n-3

PUFA ratio was associated with a lower first trimester systolic blood pressure, but no associations were observed in other trimesters.

Table 2.2.2. Associations of maternal fatty acid concentrations with blood pressure development during pregnancy[1]

Maternal fatty acids in SD	Differences in blood pressure (mmHg) in each trimester (95% confidence interval) per SD difference in maternal PUFAs concentrations					
	First trimester		Second trimester		Third trimester	
	Systolic blood pressure	Diastolic blood pressure	Systolic blood pressure	Diastolic blood pressure	Systolic blood pressure	Diastolic blood pressure
Saturated fatty acids (SFAs)						
Total SFA	1.29 (0.97, 1.61)**	0.92 (0.68, 1.17)**	1.76 (1.49, 2.04)**	1.33 (1.12, 1.55)**	1.41 (1.12, 1.69)**	0.86 (0.64, 1.07)**
Myristic acid (C14:0)	0.23 (-0.10, 0.55)	-0.12 (-0.37, 0.13)	0.97 (0.69, 1.25)**	0.31 (0.09, 0.53)**	0.75 (0.46, 1.03)**	0.18 (-0.04, 0.40)
Palmitic acid (C16:0)	0.93 (0.61, 1.25)**	0.80 (0.55, 1.05)**	1.15 (0.87, 1.43)**	0.95 (0.74, 1.17)**	0.96 (0.67, 1.24)**	0.64 (0.42, 0.86)**
Stearic acid (C18:0)	0.12 (-0.19, 0.44)	-0.04 (-0.28, 0.21)	0.24 (-0.04, 0.52)	0.16 (-0.05, 0.38)	0.11 (-0.18, 0.40)	0.09 (-0.13, 0.30)
Monounsaturated fatty acids (MUFAs)						
Total MUFAs	-0.07 (-0.42, 0.28)	-0.21 (-0.48, 0.06)	0.18 (-0.12, 0.49)	-0.07 (-0.30, 0.17)	0.20 (-0.11, 0.51)	0.15 (-0.09, 0.39)
Palmitoleic acid (C16:1n7)	0.56 (0.24, 0.88)**	0.40 (0.15, 0.65)**	0.95 (0.68, 1.24)**	0.75 (0.53, 0.97)**	0.82 (0.53, 1.10)**	0.67 (0.45, 0.89)**
Oleic acid (C18:1n9)	-0.08 (-0.42, 0.27)	-0.23 (-0.51, 0.03)	0.21 (-0.10, 0.50)	-0.11 (-0.34, 0.13)	0.19 (-0.12, 0.50)	0.09 (-0.15, 0.33)
n-3 Polyunsaturated fatty acids (PUFAs)						
Total n-3 PUFAs	0.03 (-0.30, 0.36)	-0.22 (-0.48, 0.04)	-0.40 (-0.69, -0.11)**	-0.19 (-0.42, 0.04)	-0.31 (-0.60, -0.01)*	-0.25 (-0.48, -0.02)*
α-Linolenic acid (C18:3n3)	0.08 (-0.24, 0.41)	-0.29 (-0.55, -0.03)*	0.23 (-0.05, 0.51)	-0.10 (-0.32, 0.12)	0.20 (-0.09, 0.49)	-0.04 (-0.27, 0.18)
Eicosapentaenoic acid (C20:5n3)	0.32 (-0.01, 0.64)	-0.07 (-0.32, 0.18)	0.21 (-0.08, 0.49)	0.09 (-0.14, 0.31)	0.21 (-0.08, 0.50)	-0.03 (-0.26, 0.20)
Docosahexaenoic acid (C22:6n3)	-0.06 (-0.39, 0.27)	-0.18 (-0.43, 0.08)	-0.57 (-0.86, -0.28)**	-0.25 (-0.47, -0.03)*	-0.47 (-0.76, -0.18)**	-0.26 (-0.49, -0.04)*
n-6 Polyunsaturated fatty acids (PUFAs)						
Total n-6 PUFAs	-0.65 (-1.01, -0.29)**	-0.19 (-0.48, 0.09)	-0.76 (-1.07, -0.45)**	-0.51 (-0.75, -0.27)**	-0.70 (-1.02, -0.38)**	-0.38 (-0.63, -0.14)**
Linoleic acid (C18:2n6)	-0.92 (-1.25, -0.59)**	-0.55 (-0.80, -0.29)**	-0.90 (-1.19, -0.61)**	-0.66 (-0.88, -0.44)**	-0.74 (-1.04, -0.45)**	-0.57 (-0.80, -0.35)**
Y-Linoleic acid (C18:3n6)	0.21 (-0.10, 0.52)	0.13 (-0.12, 0.37)	0.54 (0.27, 0.82)**	0.40 (0.19, 0.61)**	0.47 (0.19, 0.75)**	0.24 (0.02, 0.45)**
Arachidonic acid (C20:4n6)	0.42 (0.10, 0.75)*	0.46 (0.20, 0.71)**	0.02 (-0.26, 0.30)	0.13 (-0.09, 0.35)	-0.09 (-0.38, 0.20)	0.21 (-0.01, 0.44)
n-6/n-3 PUFAs ratio	-0.37 (-0.72, 0.02)**	0.07 (-0.20, 0.34)	0.02 (-0.28, 0.32)	-0.06 (-0.29, 0.18)	0.07 (-0.24, 0.37)	0.03 (-0.21, 0.27)

[1]Values are regression coefficients (95% Confidence Interval) and reflect the difference in blood pressure in mmHg per standard deviation change in maternal fatty acid concentrations. All models are adjusted for gestational age at enrollment and gestational age at maternal blood pressure measurement, maternal age, parity, ethnicity, education, body mass index, smoking, folic acid supplementation use, alcohol consumption, and total calorie intake during pregnancy.

Maternal fatty acid concentrations and placental vascular function

Table 2.2.3 shows that higher total SFA concentrations were not associated with second and third trimester placental vascular resistance indices. Higher total MUFA concentrations were only associated with lower second and third trimester umbilical artery pulsatility index (differences: -0.05 (95% CI:-0.09, -0.02) and -0.05 (95% CI: -0.09, -0.02) per SD increase of total MUFA concentrations). Among the individual MUFAs, higher palmitoleic and oleic acid concentrations were associated with lower second trimester umbilical artery pulsatility index and uterine artery resistance indices (all *P* values<0.05). No consistent associations were observed among n-3 PUFA concentrations and placental vascular resistance measures. Higher total n-6 PUFA concentrations were associated with higher second and third trimester uterine artery resistance index (differences: 0.06 (95% CI: 0.02, 0.10) and 0.05 (95% CI: 0.01, 0.09) per SD increase in total n-6 PUFA concentrations), but no consistent associations were observed among the individual n-6 PUFAs and placental vascular resistance measures. A higher n-6/n-3 PUFAs ratio was associated with a lower second trimester umbilical artery pulsatility index (difference: -0.04 (95% CI: -0.07, -0.01) per SD increase in n-6/n-3 PUFAs ration), but not with other placental vascular resistance measures.

Table 2.2.3. Associations of maternal fatty acid concentrations with placental vascular function[1]

Maternal fatty acids in SD	Differences in placental vascular function (95% confidence interval) per SD difference in maternal fatty acid concentrations				
	Second trimester		Third trimester		
	Umbilical artery pulsatility index	Uterine artery resistance index	Umbilical artery pulsatility index	Uterine artery resistance index	Uterine artery notching
Saturated fatty acids (SFAs)					
Total SFAs	-0.03 (-0.06, 0.01)	-0.02 (-0.05, 0.01)	-0.01 (-0.04, 0.03)	-0.02 (-0.05, 0.01)	0.01 (-0.02, 0.05)
Myristic acid (C14:0)	-0.04 (-0.07, -0.01)*	-0.02 (-0.06, 0.01)	-0.02 (-0.05, 0.01)	-0.02 (-0.06, 0.01)	-0.02 (-0.06, 0.01)
Palmitic acid (C16:0)	-0.03 (-0.06, -0.01)*	-0.04 (-0.07, -0.002)	-0.03 (-0.06, -0.001)	-0.01 (-0.04, 0.03)	-0.01 (-0.03, 0.03)
Stearic acid (C18:0)	0.03 (0.01, 0.06)*	0.02 (-0.01, 0.06)	0.03 (0, 0.05)	0.01 (-0.03, 0.04)	0.02 (-0.01, 0.05)
Monounsaturated fatty acids (MUFAs)					
Total MUFAs	-0.05 (-0.09, -0.02)**	-0.01 (-0.04, 0.02)	-0.05 (-0.09, -0.02)**	-0.01 (-0.04, 0.02)	0.01 (-0.03, 0.04)
Palmitoleic acid (C16:1n7)	-0.04 (-0.07, -0.01)*	-0.03 (-0.06, -0.01)**	-0.03 (-0.06, 0.01)	-0.01 (-0.04, 0.01)	-0.01 (-0.04, 0.03)
Oleic acid (C18:1n9)	-0.004 (-0.03, 0.03)	-0.05 (-0.09, -0.02)**	-0.02 (-0.05, 0.01)	-0.06 (-0.09, -0.02)**	0.01 (-0.03, 0.04)
n-3 Polyunsaturated fatty acids (PUFAs)					
Total n-3 PUFAs	0.03 (0.01, 0.06)*	-0.01 (-0.05, 0.02)	-0.02 (-0.05, 0.01)	-0.01 (-0.05, 0.02)	-0.02 (-0.06, 0.01)
α-Linolenic acid (C18:3n3)	0.01 (-0.02, 0.04)	-0.02 (-0.05, 0.01)	-0.01 (-0.04, 0.02)	-0.02 (-0.05, 0.02)	-0.01 (-0.04, 0.03)
Eicosapentaenoic acid (C20:5n3)	0.02 (-0.01, 0.05)	-0.01 (-0.04, 0.02)	-0.04 (-0.07, -0.01)*	0.003 (-0.03, 0.04)	-0.04 (-0.07, -0.01)*
Docosahexaenoic acid (C22:6n3)	0.03 (0.01, 0.06)*	-0.005 (-0.04, 0.03)	-0.01 (-0.04, 0.02)	-0.003 (-0.04, 0.03)	-0.01 (-0.05, 0.02)
n-6 Polyunsaturated fatty acids (PUFAs)					
Total n-6 PUFAs	-0.01 (-0.04, 0.02)	0.06 (0.02, 0.10)**	0.03 (-0.001, 0.06)	0.05 (0.01, 0.09)*	0.01 (-0.03, 0.04)
Linoleic acid (C18:2n6)	-0.01 (-0.04, 0.02)	0.04 (0, 0.07)	0.01 (-0.02, 0.04)	0.01 (-0.02, 0.05)	-0.01 (-0.04, 0.03)
Y-Linoleic acid (C18:3n6)	-0.01 (-0.04, 0.02)	0 (-0.03, 0.03)	-0.01 (-0.04, 0.02)	-0.002 (-0.03, 0.03)	-0.01 (-0.04, 0.02)
Arachidonic acid (C20:4n6)	0.03 (0, 0.06)	0.04 (0.01, 0.07)	0.03 (0.01, 0.06)*	0.06 (0.03, 0.09)**	0.03 (-0.01, 0.06)
n-6/n-3 PUFAs ratio	-0.04 (-0.07, -0.01)*	0.02 (-0.01, 0.06)	0.02 (-0.01, 0.05)	0.02 (-0.01, 0.06)	0.01 (-0.03, 0.04)

[1]Values are regression coefficients (95% Confidence Interval) and reflect the difference in placental resistance index in SDS per standard deviation change maternal fatty acid concentrations. All models are adjusted for gestational age at enrollment and gestational age at placental resistance index measurement, maternal age, parity, ethnicity, education, body mass index, smoking, alcohol consumption, folic acid supplementation use, and total calorie intake during pregnancy.

Maternal fatty acid concentrations and the risk of gestational hypertensive disorders

Table 2.2.4 shows that higher total SFA concentrations were associated with the risk of gestational hypertensive disorders, gestation hypertension and preeclampsia (OR 1.23 (95% CI: 1.09, 1.37), 1.19 (95% CI: 1.04, 1.37) and 1.27 (95% CI: 1.08, 1.51) per SD increase in total SFAs). Among the individual SFA concentrations, only stearic acid tended to be associated with the risk of gestational hypertensive disorders. No significant associations of MUFA, n-3 PUFA and n-6 PUFA concentrations with the risk of gestational hypertensive disorders were present.

Table 2.2.4. Associations of maternal fatty acid concentrations with the risk of gestational hypertensive disorders[1]

Maternal fatty acids in SD	OR for risk of gestational hypertensive disorders (95% range) per SD change in maternal fatty acid concentrations		
	Gestational hypertensive disorders	Gestational hypertension	Preeclampsia
Saturated fatty acids (SFAs)			
Total SFAs	1.23 (1.09, 1.37)*	1.19 (1.04, 1.37)*	1.27 (1.08, 1.51)*
Myristic acid (C14:0)	1.01 (0.90, 1.13)	1.06 (0.92, 1.22)	0.92 (0.77, 1.10)
Palmitic acid (C16:0)	1.10 (0.99, 1.23)	1.05 (0.92, 1.21)	1.17 (0.99, 1.39)
Stearic acid (C18:0)	1.11 (1.00, 1.24)	1.14 (0.99, 1.30)	1.08 (0.92, 1.28)
Monounsaturated fatty acids (MUFAs)			
Total MUFAs	0.98 (0.86, 1.10)	0.92 (0.79, 1.07)	1.05 (0.87, 1.27)
Palmitoleic acid (C16:1n7)	1.09 (0.98, 1.21)	1.08 (0.94, 1.23)	1.09 (0.93, 1.29)
Oleic acid (C18:1n9)	0.97 (0.86, 1.10)	0.91 (0.78, 1.06)	1.07 (0.89, 1.28)
n-3 Polyunsaturated fatty acids (PUFAs)			
Total n-3 PUFAs	0.91 (0.81, 1.02)	0.93 (0.80, 1.08)	0.88 (0.72, 1.06)
α-Linolenic acid (C18:3n3)	0.93 (0.83, 1.04)	0.89 (0.76, 1.03)	0.99 (0.83, 1.19)
Eicosapentaenoic acid (C20:5n3)	0.95 (0.84, 1.07)	1.01 (0.88, 1.16)	0.81 (0.64, 1.02)
Docosahexaenoic acid (C22:6n3)	0.92 (0.82, 1.03)	0.92 (0.79, 1.07)	0.91 (0.76, 1.09)
n-6 Polyunsaturated fatty acids (PUFAs)			
Total n-6 PUFAs	0.97 (0.86, 1.10)	1.01 (0.86, 1.19)	0.93 (0.76, 1.13)
Linoleic acid (C18:2n6)	0.98 (0.87, 1.10)	0.99 (0.85, 1.14)	0.99 (0.83, 1.18)
Y-Linoleic acid (C18:3n6)	1.00 (0.90, 1.11)	1.03 (0.90, 1.17)	0.97 (0.82, 1.15)
Arachidonic acid (C20:4n6)	0.99 (0.89, 1.11)	1.02 (0.89, 1.17)	0.94 (0.79, 1.11)
n-6/n-3 PUFAs ratio	1.08 (0.96, 1.22)	1.07 (0.92, 1.25)	1.10 (0.92, 1.32)

[1]Values are regression coefficients (95% Confidence Interval) and reflect the risk of gestational hypertensive disorders per standard deviation change maternal fatty acid concentrations. All models are adjusted for gestational age at enrollment, maternal age, parity, ethnicity, education, body mass index, smoking, alcohol consumption, folic acid supplementation use, and total calorie intake during pregnancy.

Discussion

In this population based prospective cohort study, we observed that higher maternal SFAs, but not MUFAs, were associated with a higher blood pressure from first trimester onwards and an increased risk of gestational hypertensive disorders. In contrast, higher total maternal n-3 PUFA and n-6 PUFA concentrations, especially DHA and LA, were associated with a lower systolic and diastolic blood pressure throughout pregnancy, but not with second and third trimester placental vascular resistance indices or the risk of gestational hypertensive disorders.

Methodological considerations

This study was embedded in a population-based prospective cohort among 6,823 subjects. To the best of our knowledge, the current study is the largest population-based study focused on the associations of maternal fatty acid profiles during pregnancy,

hemodynamic adaptations and gestational hypertensive disorders. The response rate at baseline for participation in the Generation R Study cohort was 61%. The non-response would lead to biased effect estimates if the associations were different between those included and not included in the analyses, but this seems unlikely. Furthermore, not all women were already enrolled in the study in first trimester. Therefore, we did not have first trimester blood pressure measurements in ~25% of the participating women. It seems unlikely that late enrolled has biased our results. We had a relative small number of women with notching, gestational hypertension and preeclampsia, which might indicate a selection towards a healthy, low-risk population and may affect the generalizability of our results. We measured a large number of fatty acid concentrations in plasma samples only once during pregnancy. Fatty acids measured in plasma may reflect a time frame of dietary intake of approximately 2 weeks and seem to be reasonable indicators for the recent intake.[33] In addition, fatty acid patterns in plasma phospholipids show a relatively high degree of tracking over time.[34,35] Therefore, fatty acid concentrations at mid-pregnancy might be a reasonable proxy of fatty acids status throughout pregnancy. Several different exposures and outcomes were studied, but since they are strongly related, we did not perform adjustment for multiple testing. Although, detailed information about a large number of potential confounding factors was available in this study, because of the observational design, residual confounding due to other socio-demographic and lifestyle-related determinants cannot be ruled out.

Interpretation of main findings

An unfavorable fatty acids profile is associated with cardiovascular disease and mortality in adult populations.[1-3] We hypothesized that during pregnancy suboptimal maternal fatty acid concentrations, either excessive or insufficient, also adversely affect gestational hemodynamic adaptations and predispose to an increased risk of gestational hypertensive disorders.[23,24,36]

It is well known that an adverse fatty acids profile is associated with increased blood pressure in non-pregnant adult populations.[37] A study among 162 healthy subjects reported that higher MUFA decrease systolic and diastolic blood pressure, but no associations were observed between SFAs and n-3 PUFAs with blood pressure.[38] Recent meta-analyses including 7 randomized control trials among adults have shown that compared with placebo, EPA and DHA, n-3 PUFAs, reduced systolic and diastolic blood pressure in all studies combined.[39] During pregnancy, few studies assessed the influence of maternal fatty acid status with blood pressure development. A prospective cohort study among 225 healthy pregnant women from Brazil showed that maternal serum concentrations of SFA, MUFA and n-6 PUFA were positively as-

sociated with systolic blood pressure measured in all trimesters during pregnancy.[40] A birth cohort study among 751 Chinese, Malay and Indian women reported that higher n-3 PUFAs at mid-pregnancy were related to lower maternal blood pressure in second trimester.[41] In the same study they have found that higher GLA, a n-6 PUFA, was marginally positively associated with systolic blood pressure, whereas linoleic acid, a n-6 PUFA, and total n-6 PUFAs showed no significant associations with blood pressure. Two small randomized controlled trials from Denmark did not show an effect of a MUFA-enriched diet, or supplementation with marine n-3 PUFA in the second half of pregnancy on blood pressure levels.[42,43] To the best of our knowledge, this is the first longitudinal study focused on multiple maternal fatty acid concentrations with repeatedly measured blood pressure throughout pregnancy. We observed that higher total SFAs, especially palmitic acid and palmitoleic acid, a MUFA, were associated with higher systolic and diastolic blood pressure from the first trimester onwards. Contrary, higher total maternal n-3 PUFA and n-6 PUFA concentrations, especially DHA and LA concentrations were associated with a lower systolic and diastolic blood pressure. Thus, in line with results from adult populations, our results suggest that an adverse maternal fatty acids profile with higher SFA concentrations and lower DHA and LA concentrations is associated with higher blood pressure levels throughout pregnancy.

Adequate placental vascular function is important during pregnancy for both maternal and fetal pregnancy outcomes. Fatty acids may influence placental angiogenesis and placental vascular function.[9,44] Thus far, animal studies have shown that a high intake of SFAs before and during pregnancy may induce placental vascular damage, increased placental oxidative stress, and loss of trophoblast cells.[45,46] It has also been suggested that maternal dietary n-3 PUFA supplementation may reduce excess inflammation and oxidative damage in the trophoblast cells.[47] A study among 77 Icelandic healthy pregnant women showed an inverse association between maternal n-3 PUFA status in first trimester of pregnancy and placental weight at birth.[48] A control clinical trial in a group of 145 patients suggested that maternal supplementation with PUFAs, especially DHA, reduces deep placentation disorders, by improving deep placentation.[49] However, the direct effect of plasma fatty acids during pregnancy on placental vascular function, assessed by Doppler ultrasound of umbilical and uterine artery blood flow during pregnancy, has not been studied. We did not observe consistent associations of total and individual fatty acids on placental vascular function. Higher concentrations of MUFA, but not SFA, tended to be associated with a lower placental vascular resistance, whereas higher concentrations of n-3 and n-6 PUFA tended to be associated with a higher placental vascular resistance. The lack of consistent associations within our study cohort may be explained by a selection towards

a relatively healthy study population. Further studies are needed to replicate our findings and to assess these associations among higher risk populations.

Several studies have reported that fatty acids profile might influence the risk of gestational hypertensive disorders.[14,15,50] Previous case-control studies have found that higher concentrations of MUFA and lower concentrations of n-3 PUFA were positively associated with the risk of preeclampsia.[14,25,51] A study among 1,718 women from Project Viva, showed that high intake of DHA and EPA, n-3 PUFAs, were associated with a lower risk of preeclampsia.[13] Furthermore, a small study among 17 women from USA showed that lower level of n-3 and n-6 PUFAs and a higher n-6/n-3 PUFAs ratio were present in placentas of women with preeclampsia compared with normotensive pregnant women.[52] We observed that higher SFAs, but not other fatty acids were associated with an increased risk of gestational hypertensive disorders. The difference with other observational studies might be due to the small number of gestational hypertensive disorder cases in our study population and differences in adjustment for possible covariates. However, our findings are in line with findings from a large multicenter trial among 27,000 women, which failed to show any protective effect of marine fatty acids on the risk for preeclampsia.[53]

The mechanism by which fatty acids may affect hemodynamic adaptations during pregnancy remains controversial. SFAs might contribute to increases in blood pressure through the development of plaques on the walls of blood vessels, which then result in the reduction of elasticity.[38] On the other hand, a n-3 and specifically LA from n-6 PUFAs are converted to prostaglandins, which reduce blood pressure by affecting arterial vasodilation, electrolyte balance, and renal release of renin or pressor hormones.[38,54] The fatty acids are the precursors of the eicosanoids with include thromboxane's. Placental abnormalities, including oxidative stress and increased vasoconstrictor generation of thromboxane by placental trophoblast cells, are considered to contribute to the pathophysiology of preeclampsia.[55,56]

Conclusion

We observed that higher maternal SFAs, but not MUFAs, were associated with a higher blood pressure, whereas higher total n-3 PUFA and n-6 PUFA concentrations, especially DHA and LA were associated with a lower systolic and diastolic blood pressure throughout pregnancy. Different fatty acids were not consistently associated with placental vascular function. Only higher maternal SFA concentrations were associated with an increased risk of gestational hypertensive disorders. Further studies are

needed to provide more insight into the role that maternal fatty acid concentrations play in the etiology and pathophysiology of gestational hypertensive disorders.

References

1. Xu J, Eilat-Adar S, Loria C, Goldbourt U, Howard BV, Fabsitz RR, Zephier EM, Mattil C and Lee ET. Dietary fat intake and risk of coronary heart disease: the Strong Heart Study. Am J Clin Nutr. 2006;84:894-902.
2. Kris-Etherton PM. AHA Science Advisory. Monounsaturated fatty acids and risk of cardiovascular disease. American Heart Association. Nutrition Committee. Circulation. 1999;100:1253-8.
3. Lemaitre RN, King IB, Mozaffarian D, Sotoodehnia N, Rea TD, Kuller LH, Tracy RP and Siscovick DS. Plasma phospholipid trans fatty acids, fatal ischemic heart disease, and sudden cardiac death in older adults: the cardiovascular health study. Circulation. 2006;114:209-15.
4. Wang L, Manson JE, Forman JP, Gaziano JM, Buring JE and Sesso HD. Dietary fatty acids and the risk of hypertension in middle-aged and older women. Hypertension. 2010;56:598-604.
5. Thies F, Garry JM, Yaqoob P, Rerkasem K, Williams J, Shearman CP, Gallagher PJ, Calder PC and Grimble RF. Association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomised controlled trial. Lancet. 2003;361:477-85.
6. Morris MC, Sacks F and Rosner B. Does fish oil lower blood pressure? A meta-analysis of controlled trials. Circulation. 1993;88:523-33.
7. Wijendran V and Hayes KC. Dietary n-6 and n-3 fatty acid balance and cardiovascular health. Annu Rev Nutr. 2004;24:597-615.
8. Calder PC. Omega-3 fatty acids and inflammatory processes. Nutrients. 2010;2:355-74.
9. Jones ML, Mark PJ and Waddell BJ. Maternal dietary omega-3 fatty acids and placental function. Reproduction. 2014;147:R143-52.
10. Lager S, Jansson T and Powell TL. Differential regulation of placental amino acid transport by saturated and unsaturated fatty acids. Am J Physiol Cell Physiol. 2014;307:C738-44.
11. Frias AE, Morgan TK, Evans AE, Rasanen J, Oh KY, Thornburg KL and Grove KL. Maternal high-fat diet disturbs uteroplacental hemodynamics and increases the frequency of stillbirth in a nonhuman primate model of excess nutrition. Endocrinology. 2011;152:2456-64.
12. Gaillard R, Arends LR, Steegers EA, Hofman A and Jaddoe VW. Second- and third-trimester placental hemodynamics and the risks of pregnancy complications: the Generation R Study. Am J Epidemiol. 2013;177:743-54.
13. Oken E, Ning Y, Rifas-Shiman SL, Rich-Edwards JW, Olsen SF and Gillman MW. Diet during pregnancy and risk of preeclampsia or gestational hypertension. Ann Epidemiol. 2007;17:663-8.
14. Mahomed K, Williams MA, King IB, Mudzamiri S and Woelk GB. Erythrocyte omega-3, omega-6 and trans fatty acids in relation to risk of preeclampsia among women delivering at Harare Maternity Hospital, Zimbabwe. Physiol Res. 2007;56:37-50.
15. Bakheit KH, Ghebremeskel K, Pol K, Elbashir MI and Adam I. Erythrocyte omega-3 and omega-6 fatty acids profile in Sudanese women with pre-eclampsia. J Obstet Gynaecol. 2010;30:151-4.
16. Zhou SJ, Yelland L, McPhee AJ, Quinlivan J, Gibson RA and Makrides M. Fish-oil supplementation in pregnancy does not reduce the risk of gestational diabetes or preeclampsia. Am J Clin Nutr. 2012;95:1378-84.

17. Jaddoe VW, van Duijn CM, Franco OH, van der Heijden AJ, van Iizendoorn MH, de Jongste JC, van der Lugt A, Mackenbach JP, Moll HA, Raat H, Rivadeneira F, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC and Hofman A. The Generation R Study: design and cohort update 2012. Eur J Epidemiol. 2012;27:739-56.
18. Kruithof CJ, Kooijman MN, van Duijn CM, Franco OH, de Jongste JC, Klaver CC, Mackenbach JP, Moll HA, Raat H, Rings EH, Rivadeneira F, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC, Wolvius EB, Hofman A and Jaddoe VW. The Generation R Study: Biobank update 2015. Eur J Epidemiol. 2014;29:911-27.
19. Declaration of Helsinki. Ethical principles for medical research involving human subjects. J Indian Med Assoc. 2009;107:403-5.
20. Glaser C, Demmelmair H and Koletzko B. High-throughput analysis of fatty acid composition of plasma glycerophospholipids. J Lipid Res. 2010;51:216-21.
21. Wang L, Manson JE, Rautiainen S, Gaziano JM, Buring JE, Tsai MY and Sesso HD. A prospective study of erythrocyte polyunsaturated fatty acid, weight gain, and risk of becoming overweight or obese in middle-aged and older women. Eur J Nutr. 2015.
22. Markey O, Vasilopoulou D, Givens DI and Lovegrove JA. Dairy and cardiovascular health: Friend or foe? Nutr Bull. 2014;39:161-171.
23. Chen X, Scholl TO, Leskiw M, Savaille J and Stein TP. Differences in maternal circulating fatty acid composition and dietary fat intake in women with gestational diabetes mellitus or mild gestational hyperglycemia. Diabetes Care. 2010;33:2049-54.
24. Jensen CL. Effects of n-3 fatty acids during pregnancy and lactation. Am J Clin Nutr. 2006;83:1452S-1457S.
25. Qiu C, Sanchez SE, Larrabure G, David R, Bralley JA and Williams MA. Erythrocyte omega-3 and omega-6 polyunsaturated fatty acids and preeclampsia risk in Peruvian women. Arch Gynecol Obstet. 2006;274:97-103.
26. Mocking RJ, Assies J, Lok A, Ruhe HG, Koeter MW, Visser I, Bockting CL and Schene AH. Statistical methodological issues in handling of fatty acid data: percentage or concentration, imputation and indices. Lipids. 2012;47:541-7.
27. El Assaad MA, Topouchian JA, Darne BM and Asmar RG. Validation of the Omron HEM-907 device for blood pressure measurement. Blood Press Monit. 2002;7:237-41.
28. Baschat AA and Hecher K. Fetal growth restriction due to placental disease. Semin Perinatol. 2004;28:67-80.
29. Verburg BO, Jaddoe VW, Wladimiroff JW, Hofman A, Witteman JC and Steegers EA. Fetal hemodynamic adaptive changes related to intrauterine growth: the Generation R Study. Circulation. 2008;117:649-59.
30. Coolman M, de Groot CJ, Jaddoe VW, Hofman A, Raat H and Steegers EA. Medical record validation of maternally reported history of preeclampsia. J Clin Epidemiol. 2010;63:932-7.
31. Brown MA, Lindheimer MD, de Swiet M, Van Assche A and Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). Hypertens Pregnancy. 2001;20:IX-XIV.
32. Royston P, Ambler G and Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. Int J Epidemiol. 1999;28:964-74.
33. Skeaff CM, Hodson L and McKenzie JE. Dietary-induced changes in fatty acid composition of human plasma, platelet, and erythrocyte lipids follow a similar time course. J Nutr. 2006;136:565-9.

34. Zeleniuch-Jacquotte A, Chajes V, Van Kappel AL, Riboli E and Toniolo P. Reliability of fatty acid composition in human serum phospholipids. Eur J Clin Nutr. 2000;54:367-72.
35. Guerra A, Demmelmair H, Toschke AM and Koletzko B. Three-year tracking of fatty acid composition of plasma phospholipids in healthy children. Ann Nutr Metab. 2007;51:433-8.
36. van Eijsden M, Hornstra G, van der Wal MF, Vrijkotte TG and Bonsel GJ. Maternal n-3, n-6, and trans fatty acid profile early in pregnancy and term birth weight: a prospective cohort study. Am J Clin Nutr. 2008;87:887-95.
37. Zeng FF, Sun LL, Liu YH, Xu Y, Guan K, Ling WH and Chen YM. Higher erythrocyte n-3 PUFAs are associated with decreased blood pressure in middle-aged and elderly Chinese adults. J Nutr. 2014;144:1240-6.
38. Rasmussen BM, Vessby B, Uusitupa M, Berglund L, Pedersen E, Riccardi G, Rivellese AA, Tapsell L, Hermansen K and Group KS. Effects of dietary saturated, monounsaturated, and n-3 fatty acids on blood pressure in healthy subjects. Am J Clin Nutr. 2006;83:221-6.
39. Miller PE, Van Elswyk M and Alexander DD. Long-chain omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid and blood pressure: a meta-analysis of randomized controlled trials. Am J Hypertens. 2014;27:885-96.
40. Dayana Farias, Fernanda Rebelo, Ilana Eshriqui, Jaqueline Lepsch, Thatiana Pinto, Ana Franco-Sena, and NL, Kac G and Maternal Serum Fatty Acids are Positively Associated with Systolic Blood Pressure Changes during Pregnancy: Longitudinal Study. The FASEB Journal. 2015;29.
41. Lim WY, Chong M, Calder PC, Kwek K, Chong YS, Gluckman PD, Godfrey KM, Saw SM, Pan A and Group GS. Relations of plasma polyunsaturated Fatty acids with blood pressures during the 26th and 28th week of gestation in women of Chinese, Malay, and Indian ethnicity. Medicine (Baltimore). 2015;94:e571.
42. Lauszus FF, Rasmussen OW, Henriksen JE, Klebe JG, Jensen L, Lauszus KS and Hermansen K. Effect of a high monounsaturated fatty acid diet on blood pressure and glucose metabolism in women with gestational diabetes mellitus. Eur J Clin Nutr. 2001;55:436-43.
43. Salvig JD, Olsen SF and Secher NJ. Effects of fish oil supplementation in late pregnancy on blood pressure: a randomised controlled trial. Br J Obstet Gynaecol. 1996;103:529-33.
44. Belkacemi L, Nelson DM, Desai M and Ross MG. Maternal undernutrition influences placental-fetal development. Biol Reprod. 2010;83:325-31.
45. Liang C, DeCourcy K and Prater MR. High-saturated-fat diet induces gestational diabetes and placental vasculopathy in C57BL/6 mice. Metabolism. 2010;59:943-50.
46. Liang C, Oest ME and Prater MR. Intrauterine exposure to high saturated fat diet elevates risk of adult-onset chronic diseases in C57BL/6 mice. Birth Defects Res B Dev Reprod Toxicol. 2009;86:377-84.
47. Jones ML, Mark PJ, Keelan JA, Barden A, Mas E, Mori TA and Waddell BJ. Maternal dietary omega-3 fatty acid intake increases resolvin and protectin levels in the rat placenta. J Lipid Res. 2013;54:2247-54.
48. Magnusardottir AR, Steingrimsdottir L, Thorgeirsdottir H, Hauksson A and Skuladottir GV. Red blood cell n-3 polyunsaturated fatty acids in first trimester of pregnancy are inversely associated with placental weight. Acta Obstet Gynecol Scand. 2009;88:91-7.
49. Carvajal JA. Docosahexaenoic acid supplementation early in pregnancy may prevent deep placentation disorders. Biomed Res Int. 2014;2014:526895.

50. Velzing-Aarts FV, van der Klis FR, van der Dijs FP and Muskiet FA. Umbilical vessels of preeclamptic women have low contents of both n-3 and n-6 long-chain polyunsaturated fatty acids. Am J Clin Nutr. 1999;69:293-8.
51. Kazemian E, Dorosty-Motlagh AR, Sotoudeh G, Eshraghian MR, Ansary S and Omidian M. Nutritional status of women with gestational hypertension compared with normal pregnant women. Hypertens Pregnancy. 2013;32:146-56.
52. Wang Y, Walsh SW and Kay HH. Placental tissue levels of nonesterified polyunsaturated fatty acids in normal and preeclamptic pregnancies. Hypertens Pregnancy. 2005;24:235-45.
53. Sibai BM. Prevention of preeclampsia: a big disappointment. Am J Obstet Gynecol. 1998;179:1275-8.
54. Farvid MS, Ding M, Pan A, Sun Q, Chiuve SE, Steffen LM, Willett WC and Hu FB. Dietary linoleic acid and risk of coronary heart disease: a systematic review and meta-analysis of prospective cohort studies. Circulation. 2014;130:1568-78.
55. Wang Y, Walsh SW and Kay HH. Placental lipid peroxides and thromboxane are increased and prostacyclin is decreased in women with preeclampsia. Am J Obstet Gynecol. 1992;167:946-9.
56. Walsh SW and Wang Y. Trophoblast and placental villous core production of lipid peroxides, thromboxane, and prostacyclin in preeclampsia. J Clin Endocrinol Metab. 1995;80:1888-93.

Supplemental Material

Figure S2.2.1. Flow chart of the participants

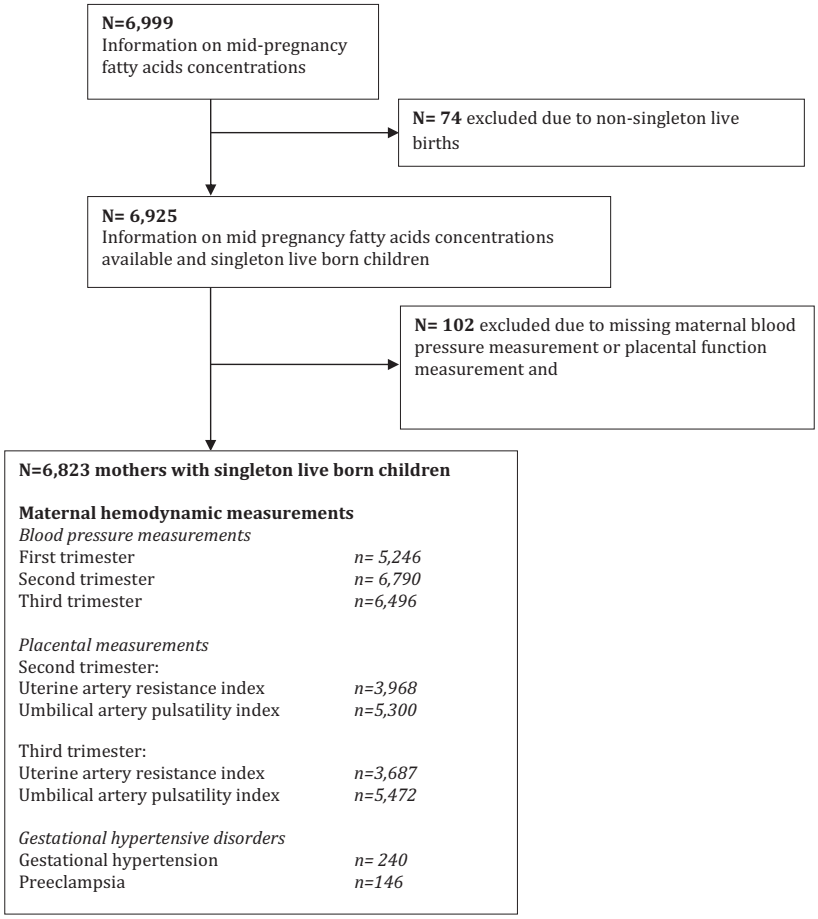


Table S2.2.1. Comparison of subject characteristics between those included and not included in the analyses

Maternal characteristics	Participation in follow-up	No participation in follow-up	P-value
Age, mean (SD), years	29.7 (5.2)	30.8 (5.3)	0.04
Height, mean (SD), (cm)	167.3 (7.4)	167.8 (7.3)	0.50
Prepregnancy body mass index, (kg/m2), mean (SD)	23.6 (4.3)	28.6 (6.7)	<0.01
Gestational age at fatty acid measures, median (95% range), (weeks)	20.7 (16.5, 24.9)	20.5 (17.1, 23.5)	0.22
Education, No. (%)			
Primary	695 (11.1)	18 (18.8)	<0.01
Secondary	2906 (46.3)	48 (50.0)	
Higher	2672 (23.5)	30 (31.3)	
Ethnicity, No. (%)			
Dutch or European	3254 (50.0)	51 (51.0)	
Non – European	3255 (50.0)	49 (49.0)	0.58
Parity, No. (%)			
Nulliparous	3791 (56.1)	53 (53.0)	0.47
Multiparous	2968 (43.9)	47 (47.0)	
Total calorie intake, kcal, mean (SD)	2043 (568)	1913 (516)	0.03
Folic acid supplement use, N. (%)			
Yes	3654 (71.6)	51 (67.1)	0.58
No	1451 (28.4)	25 (32.9)	
Smoking, No. (%)			
Yes	1125 (18.7)	20 (20.8)	0.75
No	4889 (81.3)	76 (79.2)	
Saturated fatty acids (SFAs)			
Total SFAs	695.2 (102.2)	717.9 (115.7)	0.04
Myristic acid (C14:0)	10.5 (3.6)	10.4 (3.8)	0.54
Palmitic acid (C16:0)	493.9 (76.6)	510.7 (84.3)	0.04
Stearic acid (C18:0)	185.0 (27.8)	191.3 (31.8)	0.03
Monounsaturated fatty acids (MUFAs)			
Total MUFAs	205.5 (40.1)	208.6 (45.1)	0.51
Palmitoleic acid (C16:1n7)	12.0 (5.6)	12.9 (5.7)	0.13
Oleic acid (C18:1n9)	165.7 (32.9)	167.5 (36.1)	0.66
n-3 Polyunsaturated fatty acids (PUFAs)			
Total n-3 PUFAs	103.4 (27.3)	103.9 (29.4)	0.95
α-Linolenic acid (C18:3n3)	5.0 (1.9)	4.8 (1.7)	0.24
Eicosapentaenoic acid (C20:5n3)	8..4 (5.3)	8.5 (5.7)	0.87
Docosahexaenoic acid (C22:6n3)	76.5 (20.3)	77.1 (21.8)	0.84
n-6 Polyunsaturated fatty acids (PUFAs)			
Total n-6 PUFAs	603.4 (89.1)	619.4 (96.1)	0.09
Linoleic acid (C18:2n6)	361.5 (62.9)	362.0 (61.6)	0.99
Y-Linoleic acid (C18:3n6)	1.5 (0.7)	1.5 (0.7)	0.86
Arachidonic acid (C20:4n6)	156.5 (32.6)	165.9 (42.4)	<0.01
n-6/n-3 PUFAs ratio	6.2 (1.7)	6.3 (1.7)	0.38

[1]Values represent mean (SD), median (95% range) or number of subjects (valid%). Differences were tested using Student's t-tests and Mann-Whitney tests for normally and non-normally distributed variables, respectively and χ^2-test for dichotomous variables. Abbreviations: PUFA; polyunsaturated fatty acid; SD: standard deviation.

Table S2.2.2. Correlation between all maternal fatty acid concentrations

Fatty acids	Total SFA	14:0	16:0	18:0	Total MUFA	16:1n7	18:1n9	Total n-3 PUFA	18:3n3	20:5n3	22:6n3	Total n-6 PUFA	18:2n6	20:3n6	20:4n6
Total SFA	1														
14:0	0.47**	1													
16:0	0.77**	0.33**	1												
18:0	-0.09**	-0.19**	-0.68**	1											
Total MUFA	0.15**	0.30**	0.31**	-0.37**	1										
16:1n7	0.51**	0.51**	0.59**	-0.41**	0.59**	1									
18:1n9	0.12**	0.27**	0.24**	-0.29**	0.98**	0.46**	1								
Total n-3 PUFA	-0.02	0.01	0.05**	-0.12**	0.08**	-0.02	0.05**	1							
18:3n3	0.03*	0.29**	0.06**	-0.12**	0.32**	0.16**	0.32**	0.14**	1						
20:5n3	0.09**	0.12**	0.10**	-0.09**	0.13**	0.09**	0.11**	0.76**	0.18**	1					
22:6n3	-0.06**	-0.09**	0.01	-0.09**	-0.01	-0.11**	-0.03**	0.96**	-0.01	0.60**	1				
Total n-6 PUFA	-0.47**	-0.38**	-0.52**	0.33**	-0.73**	-0.56**	-0.69**	-0.61**	-0.29**	-0.55**	-0.51**	1			
18:2n6	-0.46**	-0.28**	-0.49**	0.27**	-0.55**	-0.58**	-0.47**	-0.45**	-0.02	-0.37**	-0.39**	0.78**	1		
18:3n6	0.36**	0.43**	0.21**	0.01	0.18**	0.55**	0.15**	-0.10**	0.03**	0.00	-0.16**	-0.20**	-0.35**	1	
20:4n6	-0.12**	-0.29**	-0.14**	0.13**	-0.31**	-0.16**	-0.34**	0.00	-0.40**	-0.09**	0.09**	0.24**	-0.33**	0.08**	1

Values are Pearson correlation coefficients. Abbreviations: PUFAs: polyunsaturated fatty acids; ALA: α-Linolenic acid; EPA: Eicosapentaenoic acid; DPA: Docosapentaenoic acid; DHA: Docosahexaenoic acid; LA: Linoleic acid; GLA: Y-Linoleic acid; EDA: Eicosadienoic acid; AA: Arachidonic acid; DTA: Docosatertaenoic acid; *P-value <0.05. **P-value <0.01

Supplementary Methods

Unbalanced repeated measurements regression models. The associations of maternal fatty acid levels with repeatedly measured systolic and diastolic blood pressure were analyzed using unbalanced repeated measurement regression models. These models take the correlation between repeated measurements of the same subject into account, and allow for incomplete outcome data. Using fractional polynomials of gestational age, the best fitting models were constructed (1). For this analysis, fatty acid levels were categorized into 4 quartiles (1; 2; 3; 4) and included in these models as intercept as in interaction term with gestational age. These models can be written as:

Systolic blood pressure = $\beta_0+\beta_1$*gestational age+β_2*gestational age^{-2}+β_3*gestational age*fatty acids+β_4*fatty acids*gestational age

Diastolic blood pressure = $\beta_0+\beta_1$*gestational age+β_2*gestational age$^{0.5}$+β_3*gestational age*fatty acids+β_4*fatty acids*gestational age

In these models '$\beta_0+\beta_1$*gestational age' reflects the intercept. The intercept reflects the mean systolic and diastolic blood pressure value for these fatty acid quartiles. β_2*gestational age^{-2}+β_3*gestational age*fatty acids 'reflects the slope of change in blood pressure per week for systolic blood pressure, and 'β_2*gestational age$^{0.5}$+β_3*gestational age*fatty acids' reflects the slope of change in blood pressure per week between the different fatty acid quartiles for diastolic blood pressure. Main interest was in the term 'β_4*fatty acids*gestational age', which reflects the difference in change in blood pressure per week between the different fatty acid quartiles for systolic and diastolic blood pressure.

References

1. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. Int J Epidemiol 1999;28:964-74.

Chapter 3

Childhood outcomes

Chapter 3.1

Maternal polyunsaturated fatty acids and subcutaneous fat mass in infancy

Aleksandra Jelena Vidakovic, Susana Santos, Michelle A. Williams, Liesbeth Duijts, Albert Hofman, Hans Demmelmair, Berthold Koletzko, Vincent W.V. Jaddoe, Romy Gaillard

Adapted from Obesity (Silver Spring). 2016;24(8):1759-66.

Abstract

Objective: The associations of maternal plasma n-3 and n-6 polyunsaturated fatty acid (PUFA) concentrations during pregnancy with infant subcutaneous fat were examined.

Methods: In a population-based prospective cohort study among 904 mothers and their infants, we measured maternal plasma n-3 and n-6 PUFA concentrations at mid-pregnancy. Body mass index, total subcutaneous fat, and central-to-total subcutaneous fat ratio were calculated at 1.5, 6 and 24 months.

Results: Maternal n-3 PUFA levels were not consistently associated with infant body mass index or total subcutaneous fat. Higher maternal total n-3 PUFA levels, and specifically eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, were associated with higher central-to-total subcutaneous fat ratio at 1.5 months, whereas higher maternal total n-3 PUFA levels were associated with lower central-to-total subcutaneous fat ratio at 6 months (all *P* values< 0.05). These associations were not present at 24 months. Maternal n-6 PUFA levels were not consistently associated with infant subcutaneous fat. A higher n-6/n-3 ratio was associated with lower central-to-total subcutaneous fat ratio at 1.5 months only (*P* value<0.05).

Conclusions: Maternal n-3 PUFA levels during pregnancy may have transient effects on infant subcutaneous fat. Further studies are needed to assess the effects of maternal PUFA concentrations on fat mass development during early infancy.

Introduction

Inadequate maternal intake of polyunsaturated fatty acids (PUFA) during pregnancy may influence the risk of obesity in offspring.[1,2] Several *in vitro* and animal studies have suggested that PUFA affect the adipose tissue development during fetal and early postnatal life.[3] Among PUFA, n-3 and n-6 PUFA are of particular relevance during early life since pathways stimulated by n-6 PUFA seem to promote, while those stimulated by n-3 PUFA seem to inhibit the differentiation of adipocytes.[4,5] A US study among 1,250 mother-child pairs showed that higher maternal plasma n-3 PUFA concentrations during pregnancy tended to be associated with lower total subcutaneous fat mass in children aged 3 years.[6] In the same study, higher maternal plasma n-6/n-3 PUFA ratio was associated with higher total subcutaneous fat mass in early childhood.[6] A Dutch study among 234 mothers and their children showed that higher maternal plasma concentrations of dihomo-γ-linolenic acid (DGLA), a n-6 PUFA, during pregnancy were associated with higher total subcutaneous fat mass measured in children aged 7 years.[7] In line with these studies, we have previously reported that lower maternal n-3 PUFA concentrations and higher n-6 PUFA concentrations during pregnancy were associated with higher total body fat and abdominal fat levels at 6 years. Thus far, not much is known about the influence of maternal plasma PUFA concentrations during pregnancy on subcutaneous fat mass development throughout infancy. Infancy is a period characterized by rapid growth and subcutaneous fat mass development and is a well-known critical period for obesity and cardio-metabolic diseases later in life.[8,9] By assessing these associations in infancy further insight into the early programming effects of maternal PUFA levels on onset and timing of adiposity in the offspring can be obtained.

Therefore, we examined, in a population-based prospective cohort study from early pregnancy onwards among 904 mothers and their infants, the associations of maternal plasma n-3 and n-6 PUFA levels during pregnancy with infant subcutaneous fat mass measures.

Methods

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards among 9,778 mothers and their children living in Rotterdam, the Netherlands.[10,11] The study protocol was approved by the local Medical Ethical Committee. Written informed consent was obtained from parents.

Additional detailed assessments of fetal and infant growth and development were conducted in a subgroup of Dutch mothers and their children from late pregnancy onwards. Of all approached women, 80% agreed to participate. A total of 1,205 mothers and their singleton children participated in the subgroup study, of whom 1,083 mothers had plasma PUFA concentrations available. Of the group of 1,083 mothers and their children, 904 children had body mass index or skinfold thicknesses measured at the age of 1.5, 6 or 24 months (Flow chart is given in **Supplementary Figure S3.1.1).**

Maternal fatty acid status

Maternal non-fasting venous samples were drawn at a median gestational age of 20.5 weeks (95% range: 18.6-22.7). As previously described, EDTA plasma samples were selected and transported to the Division of Metabolic Diseases and Nutritional Medicine, Dr. von Hauner Children's Hospital, University of Munich Medical Center to analyze PUFA concentrations.[12] After being thawed, the analysis of plasma glycerophospholipid fatty acids was performed by a sensitive and precise high-throughput method, suitable in large epidemiological studies, as previously described.[13] Based on findings from previous studies, we selected maternal PUFA for our analyses, which have been associated with the risk of obesity in children and adults.[6,14] Selected maternal PUFA were total n-3 PUFA, which included α-linolenic acid (ALA, C18:3n-3), eicosapentaenoic acid (EPA, C20:5n-3), docosapentaenoic acid (DPA, C22:5n-3), and docosahexaenoic acid (DHA, C22:6n-3). Total n-6 PUFA included linoleic acid (LA, C18:2n-6), γ-linolenic acid (GLA, C18:3n-6), eicosadienoic acid (EDA, C20:2n-6), dihomo-γ-linolenic acid (DGLA, C20:3n6), arachidonic acid (AA, C20:4n-6), and docosatetraenoic acid (DTA, C22:4n-6). PUFA levels were expressed as proportion of total fatty acids present in the chromatogram (weight percentage, wt%).[15] We also calculated the ratio of total n-6/n-3 PUFA. We observed similar results when we used fatty acid concentrations in mg/L instead of percentages (results not shown).

Body fat measurements during infancy

We measured weight to the nearest gram in naked infant at the age of 1.5 and 6 months by using an electronic infant scale and at 24 months by using a mechanical personal scale (SECA, Almere, The Netherlands). Body length at the age of 1.5 and 6 months was measured in supine position to the nearest millimeter by using a neonatometer and body height at 24 months was measured in standing position by using a Harpenden stadiometer (Holtain Limited, Dyfed, UK). Body mass index (kg/m^2) was calculated and we constructed standard deviation scores based on our study sample. We observed similar results when we used age- and sex-adjusted body mass index

standard deviation scores at 24 months based on the World Health Organization Child Growth Standards (results not shown). We measured skinfold thicknesses at the ages of 1.5, 6 and 24 months on the left side of the body at the biceps, triceps, suprailiacal and subscapular area by using a skinfold caliper (Slim Guide, Creative Health Products) according to standard procedures described in detail previously. Two measurements were performed at each site and the mean was used in the analyses. Intraclass correlation coefficient among observers was 0.88 and between observers was 0.76.[16] As previously described, we calculated total subcutaneous fat mass from the sum of all four skinfold thicknesses, central subcutaneous fat mass from the sum of suprailiacal and subscapular skinfold thicknesses and peripheral subcutaneous fat mass from the sum of biceps and triceps skinfold thicknesses.[17] Measurements of body fat quantity and distribution require appropriate adjustment for body size or total fat mass, respectively, in order to undertake informative comparisons between children and within children over time. To create total subcutaneous fat mass independent of length or height and central subcutaneous fat mass independent of total subcutaneous fat mass, we estimated the optimal adjustment by log-log regression analyses.[18] Based on these analyses, total subcutaneous fat mass was only weakly correlated with length at 1.5 and 6 months or height at 24 months and was not adjusted for it, whereas a central-to-total subcutaneous fat mass ratio was calculated as central divided by total subcutaneous fat mass. The central-to-peripheral subcutaneous fat mass ratio was calculated as central divided by peripheral subcutaneous fat mass.

Covariates

We obtained information on maternal age, educational level (low, medium, high), parity (nulliparous, multiparous), prepregnancy weight, smoking habits during pregnancy (no, yes) and folic acid supplement use (no, yes) using self-reported questionnaires during pregnancy. We measured maternal height at enrolment, and calculated prepregnancy body mass index (kg/m^2). First trimester maternal nutritional information was obtained by food frequency questionnaire.[19] Information about pregnancy complications, infants' sex, gestational age and weight at birth was obtained from medical records. Gestational weight gain was calculated as the difference between maternal weight measured at 30 weeks of gestation (95% range: 28.5, 32.5) and prepregnancy weight. Information about breastfeeding duration and timing of introduction of solid foods (<3 months, 3-6 months, >6 months) was obtained by questionnaires in infancy.

Statistical analysis

We assessed the associations of maternal plasma n-3 and n-6 PUFA levels with infant adiposity measures at 1.5, 6 and 24 months and the change between these time points using linear regression models. These regression models were adjusted for gestational age at blood sampling, maternal age, educational level, parity, prepregnancy body mass index, maternal total energy intake, smoking habits, weight gain during pregnancy, folic acid supplement use, gestational diabetes, gestational hypertensive disorders, infants' sex, gestational age-adjusted birth weight, breastfeeding duration and timing of introduction of solid foods. Included covariates were selected based on their associations with the exposures and outcomes of interest in previous studies or a change in effect estimate of >10%. We constructed standard deviation scores (SDS) [(observed value - mean)/SD] for all PUFA and infant adiposity measures to enable comparison of effect estimates. We have also performed an additional analysis with central-to-peripheral subcutaneous fat mass ratio using linear regression models. In addition, we examined the associations of maternal plasma n-3 and n-6 PUFA concentrations during pregnancy with infant overweight and obesity at 24 months (body mass index above 85th percentile for age and sex) using logistic regression models. We tested for interaction terms between maternal PUFA levels and infants' sex and birth weight in relation to infant adiposity measures at 1.5, 6 and 24 months. Since no statistically significant interactions were observed, no further stratified analyses were performed. We did not adjust the main results for multiple testing because the main exposures and outcomes were correlated. However, if we would apply Bonferroni correction, we would consider a *P* value of 0.016 as significant (0.05/number of outcomes). In order to reduce potential bias associated with missing data and to maintain statistical power, we performed multiple imputations of missing covariates by generating 5 independent datasets using the Markov Chain Monte Carlo method after which the pooled effect estimates were calculated. All analyses were performed using Statistical Package for the Social Sciences version 21.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Subject characteristics

Table 3.1.1 shows the maternal and infant characteristics. Mean (SD) second trimester maternal concentrations of total glycerophospholipid bound n-3 and n-6 PUFA were 111.4 (25.8) mg/L and 592.6 (86.0) mg/L, respectively **(Table 3.1.2)**. Non-response analyses showed that as compared to mothers and infants with follow-

up measurements, mothers without follow-up measurements were slightly younger and their infant were breastfed for a shorter period (P<0.05) (**Supplementary Table S3.1.1**). Also, mothers included in the analyses had a higher total n-3 PUFA concentrations compared to those not included (**Supplementary Table S3.1.2**). Correlation coefficients between all maternal PUFA concentrations are shown in **Supplementary Table S3.1.3.**

Table 3.1.1. Characteristics of mothers and their infant (N = 904)[1]

Maternal characteristics	**Value**
Age (years), mean (SD)	31.9 (4.0)
Gestational age at PUFA measures (weeks), median (95% range)	20.5 (18.6, 22.7)
Pre-pregnancy body mass index (kg/m^2), mean (SD)	23.4 (4.1)
Gestational weight gain (kg), mean (SD)	10.0 (4.5)
Education, No. (%) higher education	574 (64.0)
Parity, No. (%) nulliparous	555 (61.4)
Total energy intake (kcal), mean (SD)	2126 (486)
Smoking during pregnancy, No. yes (%)	198 (24.1)
Folic acid supplement use, No. yes (%)	676 (90.7)
Gestational diabetes, No. (%)	10 (1.1)
Gestational hypertensive disorders, No. (%)	67 (7.6)
Infant characteristics	
Males, No. (%)	464 (51.3)
Gestational age at birth (weeks), median (95% range)	40.3 (35.8, 42.4)
Birth weight (g), mean (SD)	3509 (544)
Breastfeeding duration (months), mean (SD)	4.5 (3.8)
Introduction of solid foods n (%) >6 months	147 (18.3)
Infant adiposity characteristics	
1.5 months	
Body mass index (kg/m^2), mean (SD)	15.1 (1.4)
Total subcutaneous fat mass (mm), mean (SD)	24.0 (7.3)
Central-to-total subcutaneous fat mass ratio, mean (SD)	0.5 (0.1)
Central-to-peripheral subcutaneous fat mass ratio, mean (SD)	1.0 (0.2)
6 months	
Body mass index (kg/m^2), mean (SD)	16.8 (1.3)
Total subcutaneous fat mass (mm), mean (SD)	27.0 (6.4)
Central-to-total subcutaneous fat mass ratio, mean (SD)	0.5 (0.1)
Central-to-peripheral subcutaneous fat mass ratio, mean (SD)	0.9 (0.2)
24 months	
Body mass index (kg/m^2), mean (SD)	15.9 (1.3)
Total subcutaneous fat mass, mean (SD), mm	27.3 (7.2)
Central-to-total subcutaneous fat mass ratio, mean (SD)	0.4 (0.1)
Central-to-peripheral subcutaneous fat mass ratio, mean (SD)	0.8 (0.2)

[1]Values represent means (SDs), median (95% range) or number of subjects (valid%). Body mass index = weight/height^2. Total subcutaneous fat mass = biceps+triceps+suprailiacal+subscapular skinfold thicknesses. Central-to-total subcutaneous fat mass ratio = (suprailiacal+subscapular skinfold thicknesses)/total subcutaneous fat mass. Abbreviations: PUFA: Polyunsaturated fatty acids; SD: standard deviation.

Table 3.1.2. Second trimester maternal PUFA concentrations (N =904)[1]

	Absolute values (mg/L)	Relative values (wt%)
Total PUFA	704.1 (96.4)	42.5 (1.6)
Total n-3 PUFA	111.4 (25.8)	6.8 (1.4)
ALA	5.4 (1.6)	0.3 (0.1)
EPA	10.1 (5.3)	0.6 (0.3)
DPA	12.9 (3.9)	0.8 (0.2)
DHA	81.4 (19.4)	5.0 (1.1)
Total n-6 PUFA	592.6 (86.0)	36.3 (2.0)
LA	348.9 (59.6)	21.4 (2.5)
GLA	1.5 (0.7)	0.1 (0.0)
EDA	8.3 (1.7)	0.5 (0.1)
DGLA	63.4 (16.3)	3.9 (0.7)
AA	155.9 (31.7)	9.5 (1.4)
DTA	6.9 (2.0)	0.4 (0.1)

[1]Values represent means (SDs).
Abbreviations: ALA: α-linolenic acid; AA: arachidonic acid; DGLA dihomo-γ-linolenic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; DTA: docosatetraenoic acid; EDA: eicosadienoic acid; EPA: eicosapentaenoic acid; GLA: γ-linolenic acid; LA: linoleic acid; PUFA; polyunsaturated fatty acid.

Maternal PUFA levels and infant fat mass

Table 3.1.3 shows that maternal total n-3 PUFA levels as well as each n-3 PUFA individually were not consistently associated with infant body mass index and total subcutaneous fat mass in the adjusted models. Higher maternal total n-3 PUFA and specifically EPA, DPA, and DHA levels were associated with higher infant central-to-total subcutaneous fat mass ratio at 1.5 months (all *P* values< 0.05). However, only higher maternal total n-3 PUFA levels were associated with lower infant central-to-total subcutaneous fat mass ratio at the age of 6 months (*P* value< 0.05). Maternal n-3 PUFA levels were not associated with infant central-to-total subcutaneous fat mass ratio at 24 months. Similar results were found in the unadjusted analyses and are given in **Supplementary Table S3.1.4. Supplemental Table S3.1.5** shows that higher maternal total n-3 PUFA levels and, specifically, DPA and DHA levels were associated with a decrease in central-to-total subcutaneous fat mass ratio from 1.5 to 24 months, but no associations were found for total subcutaneous fat mass.

Table 3.1.4 shows that, in the adjusted models, higher maternal total n-6 PUFA levels were associated with a lower infant total subcutaneous fat mass at 1.5 months (*P* value<0.05), but not with infant body mass index or central-to-total subcutaneous fat mass ratio at any time points. No consistent associations of individual n-6 PUFA levels with infant adiposity measures were present at 1.5, 6 or 24 months. Similar results were found in the unadjusted analyses and are given in **Supplementary Table S3.1.6. Supplemental Table S3.1.7** shows that higher LA levels were associated

Table 3.1.3. Maternal n-3 PUFA levels and infant subcutaneous fat mass measures at 1.5, 6 and 24 months (N =904)[1-2]

	Adiposity measures at 1.5 months in SDS Difference (95% confidence interval)			Adiposity measures at 6 months in SDS Difference (95% confidence interval)			Adiposity measures at 24 months in SDS Difference (95% confidence interval)		
Maternal n-3 PUFA in SDS	**Body mass index**	**Total subcutaneous fat mass**	**Central-to-total subcutaneous fat mass ratio**	**Body mass index**	**Total subcutaneous fat mass**	**Central-to-total subcutaneous fat mass ratio**	**Body mass index**	**Total subcutaneous fat mass**	**Central-to-total subcutaneous fat mass ratio**
Total	0.02 (-0.05, 0.09)	-0.02 (-0.10, 0.05)	0.12 (0.05, 0.20)*	-0.01 (-0.09, 0.06)	-0.02 (-0.10, 0.05)	-0.08 (-0.15, -0.01)*	0.01 (-0.06, 0.09)	0.05 (-0.03, 0.13)	-0.01 (-0.09, 0.07)
ALA	0.05 (-0.01, 0.12)	-0.06 (-0.13, 0.02)	0.02 (-0.06, 0.09)	0.01 (-0.07, 0.07)	0.02 (-0.05, 0.09)	-0.03 (-0.10, 0.04)	0.02 (-0.06, 0.09)	0.01 (-0.08, 0.08)	-0.02 (-0.10, 0.05)
EPA	0.01 (-0.06, 0.07)	-0.01 (-0.08, 0.07)	0.07 (0.01, 0.15)*	-0.01 (-0.07, 0.07)	-0.03 (-0.10, 0.04)	-0.07 (-0.14, 0)	0.01 (-0.07, 0.09)	0.04 (-0.04, 0.12)	0.01 (-0.07, 0.09)
DPA	0.03 (-0.04, 0.09)	-0.13 (-0.20, -0.05)*	0.11 (0.04, 0.18)*	-0.06 (-0.12, 0.01)	-0.05 (-0.12, 0.02)	-0.02 (-0.10, 0.05)	-0.06 (-0.14, 0.01)	0.02 (-0.06, 0.09)	-0.06 (-0.13, 0.02)
DHA	0.01 (-0.06, 0.08)	0.01 (-0.07, 0.08)	0.11 (0.03, 0.18)*	-0.01 (-0.08, 0.07)	-0.01 (-0.08, 0.07)	-0.07 (-0.14, 0)	0.02 (-0.05, 0.10)	0.04 (-0.04, 0.12)	0.01 (-0.08, 0.09)

[1]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SDS of infant body mass index and fat mass measures at 1.5, 6 and 24 months per SD change in maternal n-3 PUFA levels. Body mass index = weight/height^2. Total subcutaneous fat mass = biceps+triceps+suprailiacal+subscapular skinfold thicknesses. Central-to-total subcutaneous fat mass ratio = (suprailiacal+subscapular skinfold thicknesses)/total subcutaneous fat mass.

[2]Models are adjusted for gestational age at blood sampling, maternal age, educational level, parity, pre-pregnancy body mass index, maternal total energy intake, smoking habits and weight gain during pregnancy, folic acid supplement use, gestational diabetes, gestational hypertensive disorders, infants' sex, gestational age-adjusted birth weight standard-deviation scores, breastfeeding duration and timing of introduction of solid foods (for 6 and 24 months). *P-value<0.05.

Abbreviations: ALA: α-linolenic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; PUFA; polyunsaturated fatty acid.

Table 3.1.4. Maternal n-6 PUFA levels and infant subcutaneous fat mass measures at 1.5, 6 and 24 months (N =904)[1-2]

	Adiposity measures at 1.5 months in SDS Difference (95% confidence interval)			Adiposity measures at 6 months in SDS Difference (95% confidence interval)			Adiposity measures at 24 months in SDS Difference (95% confidence interval)		
Maternal n-6 PUFA in SDS	**Body mass index**	**Total subcutaneous fat mass**	**Central-to-total subcutaneous fat mass ratio**	**Body mass index**	**Total subcutaneous fat mass**	**Central-to-total subcutaneous fat mass ratio**	**Body mass index**	**Total subcutaneous fat mass**	**Central-to-total subcutaneous fat mass ratio**
Total	0.02 (-0.05, 0.08)	-0.08 (-0.16, -0.01)*	-0.07 (-0.14, 0.01)	-0.01 (-0.07, 0.07)	0.02 (-0.05, 0.09)	0.04 (-0.04, 0.11)	-0.02 (-0.12, 0.08)	0.10 (-0.01, 0.21)	0.08 (-0.04, 0.20)
LA	0.01 (-0.05, 0.08)	-0.06 (-0.13, 0.02)	-0.03 (-0.10, 0.05)	0.01 (-0.06, 0.08)	0.05 (-0.02, 0.12)	0.03 (-0.04, 0.10)	0.04 (-0.06, 0.14)	0.02 (-0.05, 0.10)	0.03 (-0.08, 0.15)
GLA	0.02 (-0.05, 0.08)	0.04 (-0.03, 0.12)	-0.06 (-0.14, 0.01)	-0.01 (-0.08, 0.07)	-0.01 (-0.08, 0.06)	0.03 (-0.04, 0.10)	-0.06 (-0.16, 0.05)	-0.10 (-0.21, 0.02)	0.08 (-0.05, 0.20)
EDA	0.02 (-0.04, 0.09)	0.06 (-0.01, 0.13)	-0.03 (-0.10, 0.05)	0.01 (-0.06, 0.07)	-0.01 (-0.08, 0.06)	0.05 (-0.02, 0.12)	0.04 (-0.06, 0.14)	0.01 (-0.10, 0.12)	0.02 (-0.09, 0.14)
DGLA	0.02 (-0.05, 0.09)	0.07 (-0.01, 0.15)	-0.04 (-0.11, 0.04)	0.03 (-0.04, 0.10)	0.02 (-0.06, 0.09)	0.07 (0, 0.15)*	0.02 (-0.09, 0.12)	-0.02 (-0.14, 0.09)	0.04 (-0.08, 0.17)
AA	-0.01 (-0.08, 0.06)	-0.05 (-0.13, 0.02)	-0.02 (-0.09, 0.06)	-0.03 (-0.10, 0.04)	-0.07 (-0.15, 0.01)	-0.04 (-0.12, 0.03)	-0.12 (-0.22, -0.02)*	-0.05 (-0.16, 0.07)	0.01 (-0.11, 0.14)
DTA	0.01 (-0.06, 0.08)	-0.05 (-0.13, 0.03)	-0.04 (-0.12, 0.04)	-0.03 (-0.10, 0.04)	-0.01 (-0.09, 0.06)	-0.01 (-0.08, 0.07)	-0.03 (-0.14, 0.07)	-0.09 (-0.21, 0.02)	0.04 (-0.08, 0.16)

[1]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SDS of infant body mass index and subcutaneous fat mass measures at 1.5, 6 and 24 months per SD change in maternal n-6 PUFA levels. Body mass index = weight/height^2. Total subcutaneous fat mass = biceps+triceps+suprailiacal+subscapular skinfold thicknesses. Central-to-total subcutaneous fat mass ratio = (suprailiacal+subscapular skinfold thicknesses)/total subcutaneous fat mass. [2]Models are adjusted for gestational age at blood sampling, maternal age, educational level, parity, pre-pregnancy body mass index, maternal total energy intake, smoking habits and weight gain during pregnancy, folic acid supplement use, gestational diabetes, gestational hypertensive disorders, infants' sex, gestational age-adjusted birth weight standard-deviation scores, breastfeeding duration and timing of introduction of solid foods (for 6 and 24 months). *P-value<0.05. Abbreviations: AA: arachidonic acid; DGLA dihomo-γ-linolenic acid; DTA: docosatetraenoic acid; EDA: eicosadienoic acid;; GLA: γ-linolenic acid; LA: linoleic acid; PUFA; polyunsaturated fatty acid.

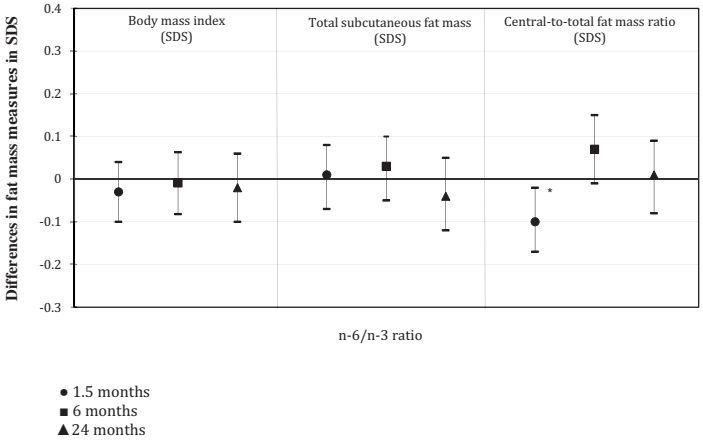
with an increase in total subcutaneous fat mass over infancy, and higher AA levels were associated with a decrease in body mass index over infancy.

We observed similar results with both maternal n-3 and n-6 PUFA when we used central-to-peripheral subcutaneous fat mass as compared to the results with central-to-total subcutaneous fat mass (**Supplementary Tables S3.1.8-S3.1.9**). Only higher maternal AA levels were associated with a lower risk of infant overweight at 24 months, but no associations were observed for the other n-3 and n-6 PUFAs with the risk of infant overweight (**Supplementary Table S3.1.10**).

Maternal n-6/n-3 PUFA ratio and infant fat mass

Figure 3.1.1 shows that higher maternal n-6/n-3 PUFA ratio was associated with lower central-to-total subcutaneous fat mass ratio at 1.5 months (difference: -0.10 (95% CI: -0.17, -0.02) SD per SD higher maternal n-6/n-3 PUFA ratio), but not with body mass index and total subcutaneous fat mass. No associations were present for maternal n-6/n-3 PUFA ratio with infant adiposity measures at 6 and 24 months.

Figure 3.1.1. Maternal n-6/n-3 PUFA ratio and infant fat mass measures at 1.5, 6 and 24 months (N =904)



Values are regression coefficients (95% Confidence Interval) that reflect the difference in SDS of infant body mass index and subcutaneous fat mass measures at 1.5, 6 and 24 months per SD change in maternal n-6/n-3 PUFA ratio. Body mass index = weight/height^2. Total subcutaneous fat mass = biceps+triceps+suprailiacal+subscapular skinfold thicknesses. Central-to-total subcutaneous fat mass ratio = (suprailiacal+subscapular skinfold thicknesses)/total subcutaneous fat mass. Models are adjusted for gestational age at blood sampling, maternal age, educational level, parity, pre-pregnancy body mass index, maternal total energy intake, smoking habits and weight gain during pregnancy, folic acid supplement use, gestational diabetes, gestational hypertensive disorders, infants' sex, gestational age-adjusted birth weight standard-deviation scores, breastfeeding duration and timing of introduction of solid foods (for 6 and 24 months). *P-value<0.05.

Discussion

In this population-based prospective cohort study, we observed that higher maternal n-3 PUFA levels were associated with a higher infant central-to-total subcutaneous fat mass ratio at 1.5 months, but with a lower infant central-to-total subcutaneous fat mass ratio at 6 months. Maternal n-3 PUFA levels were not associated with infant body mass index and total subcutaneous fat mass at any time points. Higher maternal n-6 PUFA levels were not consistently associated with subcutaneous fat mass measures during infancy.

Methodological considerations

Major strengths of this study are the population-based prospective design with detailed information on maternal PUFA concentrations and infant body fat outcomes. To the best of our knowledge, this is the largest study to date addressing the association between maternal PUFA levels during pregnancy with adiposity measures repeatedly measured in infancy. Of all mothers with available PUFA measurements, 83% (904) of mothers and children participated in the infant body fat mass measurements. The non-response could lead to biased effect estimates if the associations of interest would differ between mothers and infants included and not included in the analyses. Mothers included in the analyses had higher total n-3 PUFA concentrations compared to those not included. It is difficult to speculate if these differences might have influenced our effect estimates. We measured a large number of maternal PUFA concentrations in plasma samples once during pregnancy. No information was available about PUFA concentrations earlier or later in pregnancy. Nevertheless, PUFAs measured in plasma may reflect a time frame of dietary intake of approximately 2 weeks and seem to be reasonable indicators for the recent intake.[20] We used skinfold thickness as a measure of subcutaneous fat mass and therefore could not estimate deep fat layers, such as pre-peritoneal fat. However, during the first months of life approximately 90% of body fat is located subcutaneously and pre-peritoneal fat mass seems to increase only from the second year of life onwards.[21,22] We studied multiple infant fat mass outcomes. Since these outcomes are strongly correlated, we did not adjust our analyses for multiple testing.[23] However, a chance finding cannot be excluded. If we would apply Bonferroni correction, the associations of total n-3 PUFA levels with central-to-total fat mass at 6 months, EPA levels with central-to-total fat mass at 1.5 months, total n-6 PUFA levels with total subcutaneous fat mass at 1.5 months and DGLA levels with central-to-total at 6 months are no longer significant. Finally, although we performed an extensive adjustment for a large number of poten-

tial confounders, residual confounding, due to specific infant diet or physical activity, might still be an issue.

Interpretation of main findings

An adequate supply of n-3 and n-6 PUFA during pregnancy is important for optimal fetal and infant growth and development.[24] Rapid weight gain and increased fat mass levels during infancy may be critical for the development of adiposity in later life.[8,9,25,26] However, thus far no previous study has addressed the associations of maternal PUFA status during pregnancy with detailed fat mass measurements throughout infancy.

It has been suggested that lower maternal n-3 PUFA and higher n-6 PUFA concentrations during pregnancy are associated with higher childhood body mass index.[6,7] Fewer studies examined these associations with body mass index in infancy. A randomized double-blind controlled trial among 144 mothers and their children suggested that supplementation by DHA, a n-3 PUFA, during pregnancy and lactation reduced body mass index in late infancy.[27] However, an observational study among 244 Dutch mothers and their breastfed infant showed that n-3 and n-6 PUFA concentrations in breast milk did not affect body mass index in the first year of life.[28] In this current study, we did not observe consistent associations of maternal n-3 and n-6 PUFA concentrations during pregnancy with infant body mass index at 1.5, 6 and 24 months.

Body mass index might not be an appropriate measure of fat mass and provides limited information about body fat distribution.[29] Body fat distribution may be more strongly associated with cardio-metabolic risk factors than body mass index.[30,31] We have previously shown that maternal lower n-3 PUFA and higher n-6 PUFA concentrations during pregnancy were associated with higher total body fat and abdominal fat levels at the age of 6 years. However, not much is known about maternal PUFA concentrations with offspring fat mass outcomes at younger ages. Skinfold thicknesses can be used to estimate total and regional subcutaneous adiposity.[32] A study among 1,250 mother-child pairs in Massachusetts showed that higher DHA and EPA, n-3 PUFA concentrations from maternal diet during pregnancy and measured in cord blood, were associated with lower total subcutaneous fat mass measured at the age of 3 years.[6] In the same study, higher maternal plasma concentrations of n-6 PUFA and higher ratio of cord plasma n-6/n-3 PUFA were associated with higher total subcutaneous fat mass at the age of 3 years.[6] The INFAT randomized trial showed that the combination of an increased n-3 PUFA and a reduced AA, n-6 PUFA, dietary intake through supplementation during the perinatal period does not affect total subcutaneous fat mass during the first year of life.[33]

In our study, we observed no consistent associations of maternal n-3 PUFA concentrations with infant total subcutaneous fat mass from 1.5 months to 24 months of age. Higher maternal total n-3 PUFA and EPA, DPA and DHA concentrations were associated with higher central-to-total subcutaneous fat mass ratio at 1.5 months. However, higher total n-3 PUFA concentrations were associated with lower infant central-to-total subcutaneous fat mass ratio at 6 months. The observed associations were not explained by birth weight. Only higher maternal total n-6 PUFA concentrations were associated with lower total subcutaneous fat mass at 1.5 months, but not with other infant adiposity measures or at any other time points. Based on our findings, it seems that especially maternal n-3 PUFA may stimulate central subcutaneous fat mass development in early infancy, but this is a transient effect, which is no longer present in late infancy. The underlying mechanisms that explain the associations of maternal PUFA status during pregnancy with offspring fat mass development in infancy are not clear. It has been suggested that n-3 PUFA availability during early life leads to increased activation of peroxisome proliferator–activated receptor gamma (PPAR-γ), which has been associated with an increased deposition of subcutaneous fat mass, but not visceral fat mass, in adults.[34,35] On the other hand, it has also been suggested that n-3 PUFA levels in early life inhibit the differentiation of adipocytes, leading to lower levels of fat mass.[36] Our findings might be explained by a greater effect of n-3 PUFA on PPAR-γ and by a less apparent inhibitory effect of n-3 PUFA on the differentiation of adipocytes in early infancy. Further observational and experimental studies are needed to explore these detailed underlying mechanisms and their potential critical periods.

Conclusion

Maternal n-3 PUFA levels during pregnancy may have transient effects on infant central subcutaneous fat mass development. Maternal n-6 PUFA levels were not consistently associated with infant subcutaneous fat mass measures. Further studies are needed to assess the effects of maternal PUFA levels during pregnancy on detailed fat mass development throughout early infancy.

References

1. Grieger JA, Clifton VL. A review of the impact of dietary intakes in human pregnancy on infant birthweight. Nutrients 2015;7(1):153-178.

2. Cetin I, Alvino G, Cardellicchio M. Long chain fatty acids and dietary fats in fetal nutrition. J Physiol 2009;587(Pt 14):3441-3451.
3. Ailhaud G, Guesnet P. Fatty acid composition of fats is an early determinant of childhood obesity: a short review and an opinion. Obes Rev 2004;5(1):21-26.
4. Ailhaud G, Massiera F, Weill P, Legrand P, Alessandri JM, Guesnet P. Temporal changes in dietary fats: role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity. Prog Lipid Res 2006;45(3):203-236.
5. Kim HK, Della-Fera M, Lin J, Baile CA. Docosahexaenoic acid inhibits adipocyte differentiation and induces apoptosis in 3T3-L1 preadipocytes. J Nutr 2006;136(12):2965-2969.
6. Donahue SM, Rifas-Shiman SL, Gold DR, Jouni ZE, Gillman MW, Oken E. Prenatal fatty acid status and child adiposity at age 3 y: results from a US pregnancy cohort. Am J Clin Nutr 2011;93(4):780-788.
7. de Vries PS, Gielen M, Rizopoulos D, Rump P, Godschalk R, Hornstra G, Zeegers MP. Association between polyunsaturated fatty acid concentrations in maternal plasma phospholipids during pregnancy and offspring adiposity at age 7: the MEFAB cohort. Prostaglandins Leukot Essent Fatty Acids 2014;91(3):81-85.
8. Stettler N, Zemel BS, Kumanyika S, Stallings VA. Infant weight gain and childhood overweight status in a multicenter, cohort study. Pediatrics 2002;109(2):194-199.
9. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ 2000;320(7240):967-971.
10. Jaddoe VW, van Duijn CM, Franco OH, van der Heijden AJ, van Iizendoorn MH, de Jongste JC, van der Lugt A, Mackenbach JP, Moll HA, Raat H, Rivadeneira F, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC, Hofman A. The Generation R Study: design and cohort update 2012. Eur J Epidemiol 2012;27(9):739-756.
11. Kruithof CJ, Kooijman MN, van Duijn CM, Franco OH, de Jongste JC, Klaver CC, Mackenbach JP, Moll HA, Raat H, Rings EH, Rivadeneira F, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC, Wolvius EB, Hofman A, Jaddoe VW. The Generation R Study: Biobank update 2015. Eur J Epidemiol 2014;29(12):911-927.
12. Vidakovic AJ, Gishti O, Steenweg-de Graaff J, Williams MA, Duijts L, Felix JF, Hofman A, Tiemeier H, Jaddoe VW, Gaillard R. Higher Maternal Plasma n-3 PUFA and Lower n-6 PUFA Concentrations in Pregnancy Are Associated with Lower Childhood Systolic Blood Pressure. J Nutr 2015;145(10):2362-2368.
13. Glaser C, Demmelmair H, Koletzko B. High-throughput analysis of fatty acid composition of plasma glycerophospholipids. J Lipid Res 2010;51(1):216-221.
14. Moon RJ, Harvey NC, Robinson SM, Ntani G, Davies JH, Inskip HM, Godfrey KM, Dennison EM, Calder PC, Cooper C, Group SWSS. Maternal plasma polyunsaturated fatty acid status in late pregnancy is associated with offspring body composition in childhood. J Clin Endocrinol Metab 2013;98(1):299-307.
15. Jaddoe VW, Bakker R, van Duijn CM, van der Heijden AJ, Lindemans J, Mackenbach JP, Moll HA, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC, Hofman A. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. Eur J Epidemiol 2007;22(12):917-923.
16. Ay L, Hokken-Koelega AC, Mook-Kanamori DO, Hofman A, Moll HA, Mackenbach JP, Witteman JC, Steegers EA, Jaddoe VW. Tracking and determinants of subcutaneous fat mass in early childhood: the Generation R Study. Int J Obes (Lond) 2008;32(7):1050-1059.

17. Ketel IJ, Volman MN, Seidell JC, Stehouwer CD, Twisk JW, Lambalk CB. Superiority of skinfold measurements and waist over waist-to-hip ratio for determination of body fat distribution in a population-based cohort of Caucasian Dutch adults. Eur J Endocrinol 2007;156(6):655-661.
18. Wells JC, Cole TJ, steam As. Adjustment of fat-free mass and fat mass for height in children aged 8 y. Int J Obes Relat Metab Disord 2002;26(7):947-952.
19. Heppe DH, Medina-Gomez C, Hofman A, Franco OH, Rivadeneira F, Jaddoe VW. Maternal first-trimester diet and childhood bone mass: the Generation R Study. Am J Clin Nutr 2013;98(1):224-232.
20. Skeaff CM, Hodson L, McKenzie JE. Dietary-induced changes in fatty acid composition of human plasma, platelet, and erythrocyte lipids follow a similar time course. J Nutr 2006;136(3):565-569.
21. Olhager E, Flinke E, Hannerstad U, Forsum E. Studies on human body composition during the first 4 months of life using magnetic resonance imaging and isotope dilution. Pediatr Res 2003;54(6):906-912.
22. Holzhauer S, Zwijsen RM, Jaddoe VW, Boehm G, Moll HA, Mulder PG, Kleyburg-Linkers VA, Hofman A, Witteman JC. Sonographic assessment of abdominal fat distribution in infancy. Eur J Epidemiol 2009;24(9):521-529.
23. Bland JM, Altman DG. Multiple significance tests: the Bonferroni method. BMJ 1995;310(6973):170.
24. Koletzko B, Boey CC, Campoy C, Carlson SE, Chang N, Guillermo-Tuazon MA, Joshi S, Prell C, Quak SH, Sjarif DR, Su Y, Supapannachart S, Yamashiro Y, Osendarp SJ. Current information and Asian perspectives on long-chain polyunsaturated fatty acids in pregnancy, lactation, and infancy: systematic review and practice recommendations from an early nutrition academy workshop. Ann Nutr Metab 2014;65(1):49-80.
25. Gillman MW. Early infancy - a critical period for development of obesity. J Dev Orig Health Dis 2010;1(5):292-299.
26. Gishti O, Gaillard R, Manniesing R, Abrahamse-Berkeveld M, van der Beek EM, Heppe DH, Steegers EA, Hofman A, Duijts L, Durmus B, Jaddoe VW. Fetal and infant growth patterns associated with total and abdominal fat distribution in school-age children. J Clin Endocrinol Metab 2014;99(7):2557-2566.
27. Lucia Bergmann R, Bergmann KE, Haschke-Becher E, Richter R, Dudenhausen JW, Barclay D, Haschke F. Does maternal docosahexaenoic acid supplementation during pregnancy and lactation lower BMI in late infancy? J Perinat Med 2007;35(4):295-300.
28. Scholtens S, Wijga AH, Smit HA, Brunekreef B, de Jongste JC, Gerritsen J, Seidell JC. Long-chain polyunsaturated fatty acids in breast milk and early weight gain in breast-fed infants. Br J Nutr 2009;101(1):116-121.
29. Prentice AM, Jebb SA. Beyond body mass index. Obesity reviews : an official journal of the International Association for the Study of Obesity 2001;2(3):141-147.
30. Despres JP. Body fat distribution and risk of cardiovascular disease: an update. Circulation 2012;126(10):1301-1313.
31. Gishti O, Gaillard R, Durmus B, Abrahamse M, van der Beek EM, Hofman A, Franco OH, de Jonge LL, Jaddoe VW. BMI, total and abdominal fat distribution, and cardiovascular risk factors in school-age children. Pediatr Res 2015;77(5):710-718.
32. Wells JC, Fewtrell MS. Measuring body composition. Arch Dis Child 2006;91(7):612-617.
33. Hauner H, Much D, Vollhardt C, Brunner S, Schmid D, Sedlmeier EM, Heimberg E, Schuster T, Zimmermann A, Schneider KT, Bader BL, Amann-Gassner U. Effect of reducing the n-6:n-3 long-chain

PUFA ratio during pregnancy and lactation on infant adipose tissue growth within the first year of life: an open-label randomized controlled trial. Am J Clin Nutr 2012;95(2):383-394.

34. Deckelbaum RJ, Worgall TS, Seo T. n-3 fatty acids and gene expression. Am J Clin Nutr 2006;83(6 Suppl):1520S-1525S.
35. Laplante M, Festuccia WT, Soucy G, Gelinas Y, Lalonde J, Berger JP, Deshaies Y. Mechanisms of the depot specificity of peroxisome proliferator-activated receptor gamma action on adipose tissue metabolism. Diabetes 2006;55(10):2771-2778.
36. Ailhaud G, Guesnet P, Cunnane SC. An emerging risk factor for obesity: does disequilibrium of polyunsaturated fatty acid metabolism contribute to excessive adipose tissue development? Br J Nutr 2008;100(3):461-470.

Supplemental Material

Figure S3.1.1. Flow chart of the participants

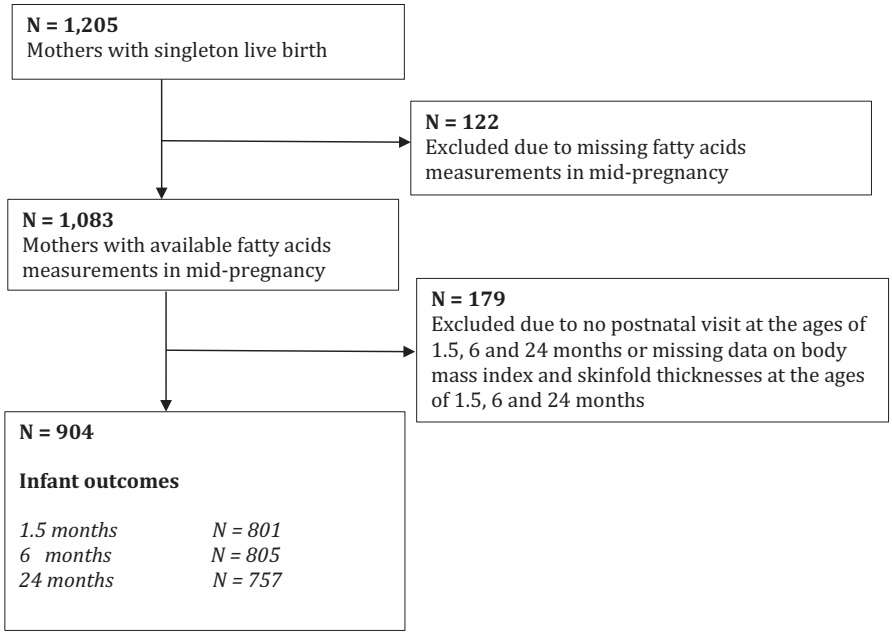


Table S3.1.1. Comparison of subject characteristics between those included and not included in the analyses (N = 1,083) [1]

Characteristics	Participation in follow-up N = 904	No participation in follow-up N = 179	P-value
Maternal characteristics			
Age (years), mean (SD)	31.9 (4.0)	30.7 (4.1)	<0.01
Gestational age at PUFA measures (weeks), median (95% range)	20.5 (18.6, 22.7)	20.5 (18.6, 22.6)	0.56
Pre-pregnancy body mass index (kg/m^2), mean (SD)	23.4 (4.1)	23.5 (4.3)	0.73
Gestational weight gain (kg), mean (SD)	10.0 (4.5)	10.4 (4.7)	0.54
Education, No. (%) higher education	574 (64.0)	102 (58.0)	0.13
Parity, No. (%) nulliparous	555 (61.4)	98 (55.4)	0.07
Total energy intake (kcal), mean (SD)	2126 (486)	2167 (497)	0.41
Smoking during pregnancy, No. yes (%)	198 (24.1)	45 (27.1)	0.22
Folic acid supplement use, No. yes (%)	676 (90.7)	139 (90.2)	0.35
Gestational diabetes, No. (%)	10 (1.1)	1 (0.6)	0.45
Gestational hypertensive disorders, No. (%)	67 (7.6)	7 (4.1)	0.15
Infant characteristics			
Males, No. (%)	464 (51.3)	98 (55.1)	0.34
Gestational age at birth (weeks), median (95% range)	40.3 (35.8, 42.4)	40.3 (34.8, 42.2)	0.66
Birth weight (g), mean (SD)	3509 (544)	3513 (591)	0.82
Breastfeeding duration (months), mean (SD)	4.5 (3.8)	3.5 (3.6)	0.01
Introduction of solid foods n (%) >6 months	147 (18.3)	11 (8.9)	0.07

[1]Values represent mean (SD), median (95% range) or number of subjects (valid%). Differences were tested using Student's t-tests and Mann-Whitney tests for normally and non-normally distributed variables, respectively and χ^2-test for dichotomous variables.
Abbreviations: PUFA: polyunsaturated fatty acid; SD: standard deviation.

Table S3.1.2. Comparison of maternal PUFA between those included and not included in the analyses (N = 1,083) [1]

Maternal PUFA	Participation in follow-up N = 904	No participation in follow-up N = 179	P-value
Total PUFA			
Absolute values (mg/L)	704.1 (95.4)	702.3 (90.7)	0.86
Percentage by weight of total sum of fatty acids (%)	42.5 (1.6)	42.3 (1.7)	0.10
Total n-3 PUFA			
Absolute values (mg/L)	111.4 (25.8)	107.5 (25.3)	0.07
Percentage by weight of total sum of fatty acids (%)	6.8 (1.4)	6.6 (1.4)	**0.01**
ALA			
Absolute values (mg/L)	5.4 (1.6)	5.3 (1.7)	0.49
Percentage by weight of total sum of fatty acids (%)	0.3 (0.1)	0.3 (0.1)	0.25
EPA			
Absolute values (mg/L)	10.1 (5.3)	9.8 (5.5)	0.45
Percentage by weight of total sum of fatty acids (%)	0.6 (0.3)	0.6 (0.3)	0.33
DPA			
Absolute values (mg/L)	12.9 (3.9)	13.2 (4.3)	0.28
Percentage by weight of total sum of fatty acids (%)	0.8 (0.2)	0.8 (0.2)	0.41
DHA			
Absolute values (mg/L)	81.5 (19.2)	77.9 (19.5)	0.02
Percentage by weight of total sum of fatty acids (%)	5.0 (1.1)	4.7 (1.1)	**<0.01**
Total n-6 PUFA			
Absolute values (mg/L)	592.6 (86.0)	595.4 (80.8)	0.45
Percentage by weight of total sum of fatty acids (%)	36.3 (2.0)	36.3 (2.0)	0.52
LA			
Absolute values (mg/L)	348.9 (59.6)	348.7 (55.2)	0.96
Percentage by weight of total sum of fatty acids (%)	21.4 (2.5)	21.3 (2.5)	0.65
GLA			
Absolute values (mg/L)	1.5 (0.7)	1.5 (0.7)	0.38
Percentage by weight of total sum of fatty acids (%)	0.1 (0.0)	0.1 (0.0)	0.46
EDA			
Absolute values (mg/L)	8.3 (1.7)	8.3 (1.7)	0.85
Percentage by weight of total sum of fatty acids (%)	0.5 (0.1)	0.5 (0.1)	0.44
DGLA			
Absolute values (mg/L)	63.3 (16.3)	64.5 (17.0)	0.22
Percentage by weight of total sum of fatty acids (%)	3.9 (0.7)	3.9 (0.7)	0.26
AA			
Absolute values (mg/L)	155.9 (31.7)	157.1 (30.9)	0.37
Percentage by weight of total sum of fatty acids (%)	9.5 (1.4)	9.6 (1.4)	0.53
DTA			
Absolute values (mg/L)	6.9 (2.0)	7.2 (2.3)	**0.01**
Percentage by weight of total sum of fatty acids (%)	0.42 (0.1)	0.4 (0.1)	**0.01**

[1]Values represent mean (SD). Differences were tested using Student's t-tests and Mann-Whitney tests for normally and non-normally distributed variables, respectively and χ^2-test for dichotomous variables. Abbreviations: ALA: α-linolenic acid; AA: arachidonic acid; DGLA dihomo-γ-linolenic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; DTA: docosatetraenoic acid; EDA: eicosadienoic acid; EPA: eicosapentaenoic acid; GLA: γ-linolenic acid; LA: linoleic acid; PUFA: polyunsaturated fatty acid.

Table S3.1.3. Correlation between all maternal fatty acid concentrations[1]

Maternal PUFA	Total PUFA	Total n-3 PUFA	ALA	EPA	DPA	DHA	Total n-6 PUFA	LA	GLA	EDA	DGLA	AA	DTA
Total PUFA	1												
Total n-3 PUFA	0.11*	1											
ALA	-0.01	0.04	1										
EPA	0.02	0.73*	0.12*	1									
DPA	-0.08*	0.56*	0.11*	0.38*	1								
DHA	0.15*	0.96*	-0.09*	0.55*	0.40*	1							
Total n-6 PUFA	0.72*	-0.61*	-0.05	-0.50*	-0.44*	-0.55*	1						
LA	0.64*	-0.40*	0.21*	-0.31*	-0.35*	-0.37*	0.76*	1					
GLA	-0.44*	-0.06	0.01	0.03	0.15*	-0.12*	-0.29*	-0.42*	1				
EDA	0.10*	-0.23*	0.19*	-0.19*	-0.18*	-0.22*	0.23*	0.34*	-0.21*	1			
DGLA	-0.37*	-0.31*	-0.03	-0.16*	-0.09*	-0.33*	-0.07	-0.28*	0.29*	0.29*	1		
AA	0.14*	0.04	-0.40*	-0.04	0.01	0.09*	0.12*	-0.46*	0.12*	-0.44*	-0.19*	1	
DTA	-0.33*	-0.21*	-0.14*	-0.20*	0.24*	-0.24**	-0.07*	-0.46*	0.37*	-0.09*	0.33*	0.38*	1

[1]Values are Pearson correlation coefficients. *P- value <0.06. Abbreviations: ALA: α-linolenic acid; AA: arachidonic acid; DGLA dihomo-γ-linolenic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; DTA: docosatetraenoic acid; EDA: eicosadienoic acid; EPA: eicosapentaenoic acid; GLA: γ-linolenic acid; LA: linoleic acid; PUFA; polyunsaturated fatty acid;

Table S3.1.4. Association of maternal n-3 PUFA levels and infant subcutaneous fat mass measures at 1.5, 6 and 24 (N =904)[1-2]

	Adiposity measures at 1.5 months in SDS Difference (95% confidence interval)			Adiposity measures at 6 months in SDS Difference (95% confidence interval)			Adiposity measures at 24 months in SDS Difference (95% confidence interval)		
Maternal n-3 PUFA in SDS	**Body mass index**	**Total subcutaneous fat mass**	**Central-to-total subcutaneous fat mass ratio**	**Body mass index**	**Total subcutaneous fat mass**	**Central-to-total subcutaneous fat mass ratio**	**Body mass index**	**Total subcutaneous fat mass**	**Central-to-total subcutaneous fat mass ratio**
Total	-0.01 (-0.07, 0.07)	-0.04 (-0.12, 0.03)	0.10 (0.03, 0.17)*	-0.04 (-0.11, 0.04)	-0.03 (-0.10, 0.04)	-0.08 (-0.15, -0.01)*	0.02 (-0.05, 0.10)	0.06 (-0.02, 0.14)	-0.03 (-0.10, 0.05)
ALA	0.04 (-0.03, 0.11)	-0.05 (-0.12, 0.03)	0.03 (-0.05, 0.10)	-0.03 (-0.10, 0.04)	0.01 (-0.06, 0.08)	-0.01 (-0.08, 0.06)	-0.03 (-0.10, 0.04)	-0.01 (-0.08, 0.07)	-0.03 (-0.10, 0.04)
EPA	-0.01 (-0.07, 0.06)	-0.02 (-0.09, 0.05)	0.07 (0.01, 0.14)*	-0.02 (-0.09, 0.06)	-0.03 (-0.10, 0.04)	-0.07 (-0.14, 0)*	0.02 (-0.06, 0.10)	0.04 (-0.04, 0.12)	-0.01 (-0.09, 0.07)
DPA	0.01 (-0.06, 0.08)	-0.13 (-0.20, -0.06)*	0.11 (0.04, 0.19)*	-0.07 (-0.14, -0.01)*	-0.06 (-0.13, 0.01)	-0.01 (-0.07, 0.07)	-0.08 (-0.15, -0.01)*	0.02 (-0.06, 0.09)	-0.07 (-0.14, 0.01)
DHA	-0.01 (-0.08, 0.06)	-0.02 (-0.09, 0.05)	0.09 (0.02, 0.16)*	-0.02 (-0.09, 0.05)	-0.02 (-0.09, 0.06)	-0.08 (-0.15, -0.01)*	0.04 (-0.03, 0.12)	0.06 (-0.02, 0.14)	-0.02 (-0.10, 0.06)

[1]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SDS of infant body mass index and subcutaneous fat mass measures from 1.5 to 24 months per SD change in maternal n-3 PUFA levels. Body mass index = weight/height^2. Total subcutaneous fat mass = biceps+triceps+suprailiacal+subscapular skinfold thicknesses. Central-to-total subcutaneous fat mass ratio = (suprailiacal+subscapular skinfold thicknesses)/total subcutaneous fat mass.

[2]Models are unadjusted for potential confounders. * P-value<0.05.

Abbreviations: AA: arachidonic acid; DGLA dihomo-γ-linolenic acid; DTA: docosatetraenoic acid; EDA: eicosadienoic acid;; GLA: γ-linolenic acid; LA: linoleic acid; PUFA; polyunsaturated fatty acid.

Table S3.1.5. Association of maternal n-3 PUFA levels and change in fat mass measures from 1.5 to 24 months (N =904)[1-2]

Maternal n-3 PUFA in SDS	Change in adiposity measures from 1.5 to 6 months in SDS Difference (95% confidence interval)			Change in adiposity measures from 1.5 to 24 months in SDS Difference (95% confidence interval)			Change in adiposity measures from 6 to 24 months in SDS Difference (95% confidence interval)		
	Body mass index	Total subcutaneous fat mass	Central-to-total subcutaneous fat mass ratio	Body mass index	Total subcutaneous fat mass	Central-to-total subcutaneous fat mass ratio	Body mass index	Total subcutaneous fat mass	Central-to-total subcutaneous fat mass ratio
Total									
Unadjusted	-0.04 (-0.11, 0.04)	0.03 (-0.07, 0.13)	0.03 (-0.07, 0.13)	0.03 (-0.07, 0.13)	0.07 (-0.04, 0.18)	-0.15 (-0.27, -0.04)*	0.07 (-0.02, 0.15)	0.04 (-0.07, 0.14)	0.06 (-0.06, 0.17)
Adjusted	-0.04 (-0.12, 0.04)	0.01 (-0.10, 0.10)	0.01 (-0.10, 0.10)	0.01 (-0.10, 0.10)	0.05 (-0.06, 0.16)	-0.16 (-0.28, -0.04)*	0.05 (-0.04, 0.13)	0.02 (-0.09, 0.13)	0.08 (-0.04, 0.20)
ALA									
Unadjusted	-0.06 (-0.13, 0.02)	-0.05 (-0.15, 0.05)	-0.05 (-0.15, 0.05)	-0.05 (-0.15, 0.05)	0.03 (-0.08, 0.14)	-0.08 (-0.19, 0.03)	0.01 (-0.07, 0.09)	-0.04 (-0.13, 0.06)	-0.06 (-0.17, 0.05)
Adjusted	-0.05 (-0.13, 0.03)	-0.03 (-0.13, 0.07)	-0.03 (-0.13, 0.07)	-0.03 (-0.13, 0.07)	0.05 (-0.06, 0.16)	-0.07 (-0.18, 0.05)	0.02 (-0.06, 0.10)	-0.04 (-0.14, 0.07)	-0.04 (-0.15, 0.07)
EPA									
Unadjusted	-0.01 (-0.09, 0.07)	0.03 (-0.07, 0.13)	0.03 (-0.07, 0.13)	0.03 (-0.07, 0.13)	0.06 (-0.06, 0.18)	-0.11 (-0.23, 0.01)	0.04 (-0.04, 0.13)	0.04 (-0.06, 0.15)	0.09 (-0.03, 0.20)
Adjusted	-0.01 (-0.08, 0.07)	0.01 (-0.09, 0.11)	0.01 (-0.09, 0.11)	0.01 (-0.09, 0.11)	0.07 (-0.05, 0.19)	-0.11 (-0.23, 0.02)	0.03 (-0.06, 0.12)	0.05 (-0.06, 0.15)	0.10 (-0.02, 0.21)
DPA									
Unadjusted	-0.09 (-0.17, 0.01)*	-0.08 (-0.18, 0.01)	-0.08 (-0.18, 0.01)	-0.08 (-0.18, 0.01)	0.11 (0.01, 0.22)*	-0.18 (-0.29, -0.07)*	0.02 (-0.07, 0.10)	0.05 (-0.06, 0.15)	-0.06 (-0.17, 0.05)
Adjusted	-0.09 (-0.17, -0.02)*	-0.08 (-0.18, 0.02)	-0.08 (-0.18, 0.02)	-0.08 (-0.18, 0.02)	0.09 (-0.02, 0.19)	-0.17 (-0.29, -0.06)*	0.02 (-0.07, 0.10)	0.03 (-0.07, 0.14)	-0.03 (-0.15, 0.09)
DHA									
Unadjusted	-0.02 (-0.10, 0.06)	0.05 (-0.05, 0.15)	0.05 (-0.05, 0.15)	0.05 (-0.05, 0.15)	0.04 (-0.07, 0.15)	-0.12 (-0.24, -0.01)*	0.07 (-0.02, 0.15)	0.03 (-0.07, 0.13)	0.07 (-0.05, 0.18)
Adjusted	-0.03 (-0.11, 0.05)	0.02 (-0.08, 0.12)	0.02 (-0.08, 0.12)	0.02 (-0.08, 0.12)	0.02 (-0.10, 0.13)	-0.13 (-0.25, -0.01)*	0.05 (-0.04, 0.13)	0 (-0.11, 0.11)	0.08 (-0.04, 0.21)

[1]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SDS of infants body mass index and subcutaneous fat mass measures from 1.5 to 24 months per SD change in maternal n-3 PUFA levels. Body mass index = weight/height^2. Total subcutaneous fat mass = biceps+triceps+suprailiacal+subscapular skinfold thicknesses. Central-to-total subcutaneous fat mass ratio = (suprailiacal+subscapular skinfold thicknesses)/total subcutaneous fat mass. [2]Adjusted model includes gestational age at blood sampling, maternal age, educational level, parity, pre-pregnancy body mass index, maternal total energy intake, smoking habits and weight gain during pregnancy, folic acid supplement use, gestational diabetes, gestational hypertensive disorders, infants' sex, gestational age-adjusted birth weight standard-deviation scores, breastfeeding duration and timing of introduction of solid foods. * P-value<0.05. Abbreviations: ALA: α-linolenic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; PUFA; polyunsaturated fatty acid.

Table S3.1.6. Association of maternal n-6 PUFA levels and infant subcutaneous fat mass measures at 1.5, 6 and 24 months (N =904)[1-2]

Maternal n-6 PUFA in SDS		Adiposity measures at 1.5 months in SDS Difference (95% confidence interval)			Adiposity measures at 6 months in SDS Difference (95% confidence interval)			Adiposity measures at 24 months in SDS Difference (95% confidence interval)		
		Body mass index	Total subcutaneous fat mass	Central-to-total subcutaneous fat mass ratio	Body mass index	Total subcutaneous fat mass	Central-to-total subcutaneous fat mass ratio	Body mass index	Total subcutaneous fat mass	Central-to-total subcutaneous fat mass ratio
Total		0.20 (-0.05, 0.09)	-0.07 (-0.14, 0.00)	-0.06 (-0.13, 0.01)	-0.01 (-0.08, 0.06)	0.01 (-0.06, 0.08)	0.04 (-0.03, 0.11)	-0.03 (-0.13, 0.06)	0.09 (-0.02, 0.20)	0.08 (-0.04, 0.19)
	LA	-0.01 (-0.08, 0.06)	-0.05 (-0.12, 0.02)	-0.03 (-0.10, 0.04)	-0.03 (-0.10, 0.04)	0.03 (-0.04, 0.10)	0.03 (-0.04, 0.10)	0.03 (-0.06, 0.14)	0.08 (-0.03, 0.19)	0.03 (-0.09, 0.14)
	GLA	0.02 (-0.05, 0.09)	0.03 (-0.04, 0.11)	-0.06 (-0.14, 0.01)	0.02 (-0.05, 0.09)	0.01 (-0.07, 0.07)	0.04 (-0.03, 0.11)	-0.07 (-0.17, 0.03)	-0.09 (-0.20, 0.03)	0.08 (-0.04, 0.20)
	EDA	0.02 (-0.05, 0.09)	0.07 (-0.01, 0.14)	-0.02 (-0.09, 0.06)	-0.01 (-0.08, 0.06)	-0.01 (-0.08, 0.06)	0.05 (-0.02, 0.12)	0.03 (-0.07, 0.13)	-0.02 (-0.13, 0.09)	0.02 (-0.09, 0.13)
	DGLA	0.06 (-0.01, 0.13)	0.08 (0.01, 0.15)*	-0.02 (-0.09, 0.05)	0.08 (0.01, 0.15)*	0.04 (-0.03, 0.11)	0.06 (-0.01, 0.13)	0.01 (-0.08, 0.11)	-0.03 (-0.14, 0.09)	0.06 (-0.06, 0.17)
	AA	0.01 (-0.06, 0.08)	-0.06 (-0.13, 0.01)	-0.01 (-0.08, 0.06)	-0.01 (-0.07, 0.07)	-0.05 (-0.12, 0.02)	-0.03 (-0.10, 0.04)	-0.11 (-0.21, -0.01)*	0.02 (-0.09, 0.12)	0.02 (-0.09, 0.13)
	DTA	0.05 (-0.02, 0.12)	-0.02 (-0.09, 0.05)	-0.02 (-0.10, 0.05)	0.01 (-0.07, 0.07)	-0.01 (-0.08, 0.06)	0.01 (-0.06, 0.08)	-0.10 (-0.20, 0.01)	-0.09 (-0.20, 0.02)	0.04 (-0.07, 0.16)

[1]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SDS of infant body mass index and subcutaneous fat mass measures from 1.5 to 24 months per SD change in maternal n-6 PUFA levels. Body mass index = weight/height^2. Total subcutaneous fat mass = biceps+triceps+suprailiacal+subscapular skinfold thicknesses. Central-to-total subcutaneous fat mass ratio = (suprailiacal+subscapular skinfold thicknesses)/total subcutaneous fat mass.

[2]Models are unadjusted for potential confounders. *P-value<0.05.

Abbreviations: AA: arachidonic acid; DGLA dihomo-γ-linolenic acid; DTA: docosatetraenoic acid; EDA: eicosadienoic acid;; GLA: γ-linolenic acid; LA: linoleic acid; PUFA; polyunsaturated fatty acid.

Table S3.1.7. Association of maternal n-6 PUFA levels and change in fat mass measures from 1.5 to 24 months (N =904)[1-2]

	Change in adiposity measures from 1.5 to 6 months in SDS Difference (95% confidence interval)			Change in adiposity measures from 1.5 to 24 months in SDS Difference (95% confidence interval)			Change in adiposity measures from 6 to 24 months in SDS Difference (95% confidence interval)		
Maternal n-6 PUFA in SDS	**Body mass index**	**Total subcutaneous fat mass**	**Central-to-total subcutaneous fat mass ratio**	**Body mass index**	**Total subcutaneous fat mass**	**Central-to-total subcutaneous fat mass ratio**	**Body mass index**	**Total subcutaneous fat mass**	**Central-to-total subcutaneous fat mass ratio**
Total									
Unadjusted	-0.02 (-0.09, 0.06)	0.07 (-0.03, 0.17)	0.09 (-0.01, 0.19)	-0.03 -0.13, 0.06)	0.09 -0.02, 0.20)	0.08 -0.04, 0.19)	-0.02 (-0.10, 0.07)	0.05 (-0.05, 0.15)	-0.04 (-0.16, 0.07)
Adjusted	-0.01 (-0.09, 0.07)	0.09 (-0.01, 0.19)	0.10 (-0.01, 0.20)	-0.02 -0.12, 0.08)	0.10 -0.01, 0.21)	0.08 -0.04, 0.20)	-0.02 (-0.10, 0.07)	0.05 (-0.06, 0.15)	-0.05 (-0.17, 0.07)
LA									
Unadjusted	-0.01 (-0.09, 0.06)	0.09 (-0.01, 0.18)	0.05 (-0.04, 0.15)	0.03 -0.06, 0.14)	0.08 -0.03, 0.19)	0.03 -0.09, 0.14)	0.04 (-0.05, 0.12)	0.04 (-0.07, 0.14)	-0.05 (-0.16, 0.06)
Adjusted	0 (-0.08, 0.08)	0.12 (0.02, 0.22)*	0.06 (-0.05, 0.16)	0.04 -0.06, 0.14)	0.12 0.01, 0.23)*	0.03 -0.08, 0.15)	0.03 (-0.06, 0.11)	0.04 (-0.07, 0.14)	-0.03 (-0.15, 0.09)
GLA									
Unadjusted	-0.02 (-0.10, 0.05)	-0.06 (-0.16, 0.05)	0.11 (0.01, 0.21)*	-0.07 -0.17, 0.03)	-0.09 -0.20, 0.03)	0.08 -0.04, 0.20)	-0.04 (-0.12, 0.04)	-0.06 (-0.16, 0.05)	-0.06 (-0.17, 0.05)
Adjusted	-0.04 (-0.12, 0.04)	-0.08 (-0.18, 0.03)	0.10 (-0.01, 0.21)	-0.06 -0.16, 0.05)	-0.10 -0.21, 0.02)	0.08 -0.05, 0.20)	-0.01 (-0.10, 0.07)	-0.04 (-0.14, 0.07)	-0.06 (-0.17, 0.06)
EDA									
Unadjusted	-0.02 (-0.10, 0.06)	-0.08 (-0.18, 0.02)	0.05 (-0.05, 0.15)	0.03 -0.07, 0.13)	-0.02 -0.13, 0.09)	0.02 -0.09, 0.13)	0.08 (-0.01, 0.16)	0.06 (-0.04, 0.16)	-0.12 (-0.23, -0.01)*
Adjusted	-0.01 (-0.08, 0.08)	-0.06 (-0.16, 0.03)	0.06 (-0.05, 0.16)	0.04 -0.06, 0.14)	0.01 -0.10, 0.12)	0.02 -0.09, 0.14)	0.08 (-0.01, 0.16)	0.08 (-0.02, 0.18)	-0.12 (-0.23, -0.01)*
DGLA									
Unadjusted	0.01 (-0.07, 0.08)	-0.04 (-0.14, 0.06)	0.08 (-0.02, 0.18)	0.01 -0.08, 0.11)	-0.03 -0.14, 0.09)	0.06 -0.06, 0.17)	0.01 (-0.07, 0.09)	0.04 (-0.06, 0.14)	-0.07 (-0.18, 0.04)
Adjusted	0.01 (-0.07, 0.08)	-0.05 (-0.15, 0.06)	0.10 (-0.01, 0.20)	0.02 -0.09, 0.12)	-0.02 -0.14, 0.09)	0.04 -0.08, 0.17)	0.03 (-0.06, 0.11)	0.06 (-0.05, 0.17)	-0.13 (-0.25, -0.01)*
AA									
Unadjusted	-0.01 (-0.08, 0.07)	-0.02 (-0.12, 0.07)	-0.02 (-0.12, 0.08)	-0.11 -0.21, -0.01)*	0.02 -0.09, 0.12)	0.02 -0.09, 0.13)	-0.09 (-0.17, -0.01)*	-0.01 (-0.11, 0.09)	0.06 (-0.05, 0.17)
Adjusted	-0.03 (-0.11, 0.05)	-0.06 (-0.16, 0.04)	-0.03 (-0.13, 0.08)	-0.12 -0.22, -0.02)*	-0.05 -0.16, 0.07)	0.01 -0.11, 0.14)	-0.09 (-0.18, -0.01)*	-0.02 (-0.13, 0.08)	0.05 (-0.07, 0.17)
DTA									
Unadjusted	-0.03 (-0.11, 0.04)	-0.02 (-0.12, 0.07)	0.05 (-0.05, 0.15)	-0.10 -0.20, 0.01)	-0.09 -0.20, 0.02)	0.04 -0.07, 0.16)	-0.01 (-0.10, 0.07)	-0.09 (-0.19, 0.01)	-0.01 (-0.12, 0.11)
Adjusted	-0.03 -0.11, 0.05)	-0.01 -0.11, 0.09)	0.06 -0.05, 0.16)	-0.03 -0.14, 0.07)	-0.09 -0.21, 0.02)	0.04 -0.08, 0.16)	0.03 -0.06, 0.11)	-0.08 -0.19, 0.03)	-0.01 -0.13, 0.11)

[1]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SDS of infants body mass index and subcutaneous fat mass measures from 1.5 to 24 months per SD change in maternal n-6 PUFA levels. Body mass index = weight/height^2. Total subcutaneous fat mass = biceps+triceps+suprailiacal+subscapular skinfold thicknesses. Central-to-total subcutaneous fat mass ratio = (suprailiacal+subscapular skinfold thicknesses)/total subcutaneous fat mass. [2]Adjusted model includes gestational age at blood sampling, maternal age, educational level, parity, pre-pregnancy body mass index, maternal total energy intake, smoking habits and weight gain during pregnancy, folic acid supplement use, gestational diabetes, gestational hypertensive disorders, infants' sex, gestational age-adjusted birth weight standard-deviation scores, breastfeeding duration and timing of introduction of solid foods. * P-value<0.05. Abbreviations: AA: arachidonic acid; DGLA dihomo-γ-linolenic acid; DTA: docosatetraenoic acid; EDA: eicosadienoic acid;; GLA: γ-linolenic acid; LA: linoleic acid; PUFA; polyunsaturated fatty acid.

Table S3.1.8. Association of maternal n-6 PUFA levels and change in fat mass measures from 1.5 to 24 months (N =904)[1-2]

	Central-to-peripheral subcutaneous fat mass ratio in SDS Difference (95% confidence interval)					
Maternal n-3 PUFA in SDS	**1.5 months**	**6 months**	**24 months**	**Change in fat mass measures from 1.5 to 6 months**	**Change in fat mass measures from 1.5 to 24 months**	**Change in fat mass measures from 6 to 24 months**
Total	0.13 (0.05, 0.20)*	-0.06 (-0.14, 0.01)	0.01 (-0.08, 0.08)	-0.20 (-0.31, -0.09)*	-0.16 (-0.29, -0.04)*	0.08 (-0.05, 0.20)
ALA	0.02 (-0.06, 0.09)	-0.03 (-0.10, 0.04)	-0.03 (-0.11, 0.05)	-0.05 (-0.16, 0.06)	-0.08 (-0.19, 0.04)	-0.05 (-0.16, 0.07)
EPA	0.08 (0.01, 0.15)*	-0.06 (-0.13, 0.01)	0.01 (-0.07, 0.09)	-0.07 (-0.27, 0.14)	-0.07 (-0.30, 0.17)	0.09 (-0.15, 0.32)
DPA	0.12 (0.05, 0.19)*	-0.01 (-0.08, 0.06)	-0.05 (-0.13, 0.03)	-0.12 (-0.22, -0.02)*	-0.18 (-0.30, -0.07)*	-0.04 (-0.15, 0.08)
DHA	0.11 (0.04, 0.19)*	-0.06 (-0.13, 0.02)	0.01 (-0.07, 0.10)	-0.20 (-0.30, -0.08)*	-0.14 (-0.26, -0.01)*	0.09 (-0.04, 0.21)

[1]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SDS in central-to-peripheral subcutaneous fat mass ratio at 1.5, 6 and 24 months per SD change in maternal n-3 PUFA levels. Central-to-peripheral subcutaneous fat mass ratio = (suprailiacal+subscapular skinfold thicknesses)/peripheral subcutaneous fat mass. [2]Models are adjusted for gestational age at blood sampling, maternal age, educational level, parity, pre-pregnancy body mass index, maternal total energy intake, smoking habits and weight gain during pregnancy, folic acid supplement use, gestational diabetes, gestational hypertensive disorders, infants' sex, gestational age-adjusted birth weight standard-deviation scores, breastfeeding duration and timing of introduction of solid foods (for 6 and 24 months). * P-value<0.05. Abbreviations: ALA: α-linolenic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; PUFA; polyunsaturated fatty acid.

Table S3.1.9. Association of maternal n-6 PUFA levels and change in fat mass measures from 1.5 to 24 months (N =904)[1-2]

	Central-to-peripheral subcutaneous fat mass ratio in SDS Difference (95% confidence interval)					
Maternal n-6 PUFA in SDS	**1.5 months**	**6 months**	**24 months**	**Change in fat mass measures from 1.5 to 6**	**Change in fat mass measures from 1.5 to 24 months**	**Change in fat mass measures from 6 to 24 months**
Total	-0.07 (-0.14, 0.01)	0.03 (-0.04, 0.10)	-0.01 (-0.09, 0.07)	0.11 (0.01, 0.21)*	0.09 (-0.04, 0.21)	-0.03 (-0.15, 0.08)
LA	-0.03 (-0.10, 0.05)	0.03 (-0.04, 0.10)	0.01 (-0.07, 0.09)	0.06 (-0.05, 0.16)	0.04 (-0.08, 0.16)	-0.02 (-0.14, 0.10)
GLA	-0.06 (-0.14, 0.01)	0.02 (-0.05, 0.09)	-0.02 (-0.10, 0.06)	0.09 (-0.01, 0.20)	0.07 (-0.05, 0.19)	-0.06 (-0.18, 0.06)
EDA	-0.02 (-0.10, 0.05)	0.05 (-0.03, 0.12)	-0.05 (-0.12, 0.03)	0.05 (-0.05, 0.16)	0.03 (-0.09, 0.14)	-0.12 (-0.23, -0.01)*
DGLA	-0.04 (-0.11, 0.04)	0.06 (-0.01, 0.14)	-0.06 (-0.14, 0.03)	0.10 (-0.01, 0.20)	0.04 (-0.08, 0.16)	-0.13 (-0.25, -0.01)*
AA	-0.02 (-0.10, 0.05)	-0.04 (-0.11, 0.04)	0.01 (-0.08, 0.08)	-0.02 (-0.13, 0.09)	0.02 (-0.10, 0.14)	0.06 (-0.06, 0.18)
DTA	-0.04 (-0.12, 0.03)	-0.01, -0.08, 0.07)	-0.05 (-0.13, 0.03)	0.06 (-0.05, 0.17)	0.04 (-0.08, 0.17)	-0.10 (-0.13, 0.11)

[1]Values are regression coefficients (95% Confidence Interval) that reflect the difference in SDS in central-to-peripheral subcutaneous fat mass ratio at 1.5, 6 and 24 months per SD change in maternal n-6 PUFA levels. Central-to-peripheral subcutaneous fat mass ratio = (suprailiacal+subscapular skinfold thicknesses)/peripheral subcutaneous fat mass.

[2]Models are adjusted for gestational age at blood sampling, maternal age, educational level, parity, pre-pregnancy body mass index, maternal total energy intake, smoking habits and weight gain during pregnancy, folic acid supplement use, gestational diabetes, gestational hypertensive disorders, infants' sex, gestational age-adjusted birth weight standard-deviation scores, breastfeeding duration and timing of introduction of solid foods (for 6 and 24 months). * P-value<0.05.

Abbreviations: AA: arachidonic acid; DGLA dihomo-γ-linolenic acid; DTA: docosatetraenoic acid; EDA: eicosadienoic acid;; GLA: γ-linolenic acid; LA: linoleic acid; PUFA; polyunsaturated fatty acid.

Table S3.1.10. Maternal n-6 PUFA levels and infant subcutaneous fat mass measures at 1.5, 6 and 24 months (N =904)[1-2]

Maternal PUFAs in SDS	Overweight risk at 24 months Odds Ratio (95% CI)[1]
Total n-3 PUFA	1.06 (0.84, 1.33)
ALA	1.12 (0.90, 1.39)
EPA	1.08 (0.86, 1.34)
DPA	0.86 (0.67, 1.09)
DHA	1.07 (0.85, 1.35)
Total n-6 PUFA	1.04 (0.83, 1.30)
LA	1.20 (0.10, 1.49)
GLA	0.89 (0.71, 1.14)
EDA	1.22 (0.10, 1.49)
DGLA	1.18 (0.94, 1.47)
AA	0.74 (0.59, 0.92)*
DTA	0.82 (0.65, 1.05)

[1]Values are odds ratio (95% Confidence Interval) that reflect the risk of overweight at 24 months per SD change in maternal n-3 and n-6 PUFA levels.

Models are adjusted for gestational age at blood sampling, maternal age, educational level, parity, pre-pregnancy body mass index, maternal total energy intake, smoking habits and weight gain during pregnancy, folic acid supplement use, gestational diabetes, gestational hypertensive disorders, infants' sex, gestational age-adjusted birth weight standard-deviation scores, breastfeeding duration and timing of introduction of solid foods. *P-value<0.05.

Abbreviations: AA: arachidonic acid; DGLA dihomo-γ-linolenic acid; DTA: docosatetraenoic acid; EDA: eicosadienoic acid;; GLA: γ-linolenic acid; LA: linoleic acid; PUFA; polyunsaturated fatty acid.

Chapter 3.2

Maternal polyunsaturated fatty acids and childhood adiposity outcomes

Aleksandra Jelena Vidakovic, Olta Gishti, Trudy Voortman, Janine F. Felix, Michelle A. Williams, Albert Hofman, Hans Demmelmair, Berthold Koletzko, Henning Tiemeier, Vincent W.V. Jaddoe, Romy Gaillard

Adapted from Am J Clin Nutr. 2016;103(4):1017-25.

Abstract

Background: Maternal polyunsaturated fatty acid (PUFA) concentrations during pregnancy may have persistent effects on growth and adiposity in the offspring. A suboptimal maternal diet during pregnancy might lead to fetal cardio-metabolic adaptations with persistent consequences in the offspring.

Objective: We examined the associations of maternal PUFA concentrations during pregnancy with childhood general and abdominal fat-distribution measures.

Design: In a population-based prospective cohort study among 4,830 mothers and their children, we measured maternal second trimester plasma n-3 and n-6 PUFA concentrations. At the median age of 6.0 years (95% range: 5.6–7.9), we measured childhood body mass index, fat mass percentage, android/gynoid fat ratio with dual-energy x-ray absorptiometry, and pre-peritoneal abdominal fat area with ultrasound. Analyses were adjusted for maternal and childhood sociodemographic- and lifestyle-related characteristics.

Results: We observed that higher maternal total n-3 PUFA concentrations, and specifically those of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA) concentrations, were associated with lower childhood total body fat percentage and lower android/gynoid fat mass ratio (*P* values<0.05), but not with childhood body mass index and abdominal pre-peritoneal fat mass area. Higher maternal total n-6 PUFA concentrations and specifically dihomo-γ-linolenic acid (DGLA) were associated with a higher childhood total body fat percentage, android/gynoid fat mass ratio and abdominal pre-peritoneal fat mass area (*P* values<0.05), but not with childhood body mass index. In line with these findings, a higher maternal n-6/n-3 PUFA ratio was associated with higher childhood total body and abdominal fat mass levels.

Conclusions: Lower maternal n-3 PUFA concentrations and higher n-6 PUFA concentrations during pregnancy are associated with higher total body fat and abdominal fat levels in childhood. Further studies are needed to replicate these observations and to explore the causality, the underlying pathways and long-term cardio-metabolic consequences.

Introduction

Fetal life and infancy are critical periods for the development of obesity in later life.[1] Maternal and fetal nutrition might affect fetal and childhood growth and the risk of obesity in childhood and adulthood. Polyunsaturated fatty acid (PUFA) are critical nutrients for fetal development.[2] Animal studies suggest that supplementing the maternal diet during pregnancy and lactation with n-3 PUFAs leads to lower offspring body weight and less fat accumulation.[3] In humans, a study among 293 mother-offspring pairs suggested that lower maternal n-3 PUFA and higher n-6 PUFA concentrations during pregnancy were associated with a higher body mass index and with a higher fat mass in children aged 4 to 6 years.[4] Furthermore, higher maternal concentrations of dihomo-γ-linolenic acid (DGLA), an n-6 PUFA, were associated with a higher childhood body mass index, waist circumference and sum of skinfolds among 234 children aged 7 years.[5] The majority of previous studies investigating relationships between early life fatty acid status and childhood obesity have used body mass index. However, body mass index does not distinguish lean mass from fat mass and provides no insight in body fat distribution.[6,7] Studies among adults and children have suggested that higher total body fat and abdominal fat mass levels are, independent from body mass index, associated with cardiovascular risk factors, disease and mortality.[8-10] Despite many previous observational and small experimental studies, it is still unclear whether maternal PUFA status influences offspring growth and fat mass development.[4,5,11-13] Thus far, results from observational studies and randomized controlled trials are not consistent.[14,15]

Therefore, we examined, in a population-based prospective cohort study from early pregnancy onwards among 4,830 mothers and their children, the associations of maternal n-3 and n-6 PUFA concentrations during pregnancy with childhood body mass index and specific body fat measures, including fat mass percentage and android/gynoid fat ratio measured by dual-energy x-ray absorptiometry (DXA) and pre-peritoneal abdominal fat area measured by ultrasound. Additionally, we examined whether these associations were independent of maternal and childhood socio-demographic and lifestyle-related characteristics.

Methods

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from foetal life to adulthood in Rotterdam, the Netherlands.[16,17]

The study has been approved by the Medical Ethical Committee of the Erasmus MC, University Medical Center in Rotterdam (MEC 198.782/2001/31). All mothers gave written consent. Pregnant women with an expected delivery date from April 2002 to January 2006 were enrolled in the study. In total, 8,879 mothers were enrolled during pregnancy, of whom 7,072 had information about PUFA concentrations available and 6,925 gave birth to singleton live born children. Childhood follow up data were available in 4,830 of these children (Flow chart is given in **Supplemental Figure S3.2.1**).

Maternal fatty acid status

Maternal venous samples were drawn at a median gestational age of 20.5 weeks (95% range: 16.5, 24.9). To analyze PUFA concentrations, EDTA plasma samples were selected and transported to the Division of Metabolic Diseases and Nutritional Medicine, Dr. von Hauner Children's Hospital, University of Munich Medical Center. After being thawed, the analysis of plasma glycerophospholipid PUFAs composition was performed by a sensitive and precise high-throughput method, suitable in large epidemiological studies, as previously described.[18] PUFA concentrations were expressed as proportion of total fatty acids present in the chromatogram (weight percentage, wt%).[19,20] Based on findings from previous studies, we selected maternal PUFAs for our analyses which have been associated with the risk of cardiovascular and metabolic outcomes in adults and maternal and fetal pregnancy outcomes.[4,21-24] Selected maternal PUFAs were: total n-3 PUFA concentrations, which included α-linolenic acid (ALA, C18:3n-3), eicosapentaenoic acid (EPA, C20:5n-3), docosapentaenoic acid (DPA, C22:5n-3), docosahexaenoic acid (DHA, C22:6n-3). Total n-6 PUFA concentrations included linoleic acid (LA, C18:2n-6), γ-linolenic acid (GLA, C18:3n-6), eicosadienoic acid (EDA, C20:2n-6), dihomo-γ-linolenic acid (DGLA, C20:3n6), arachidonic acid (AA, C20:4n-6), docosatetraenoic acid (DTA, C22:4n-6). The n-6/n-3 ratio was calculated as the sum of all n-6 PUFA (LA, GLA, EDA, DGLA and AA) divided by the sum of all n-3 PUFA (ALA, EPA, DPA and DHA).

Childhood fat distribution outcomes

At a median age of 6 years, height and weight without shoes and heavy clothing were measured. Height was measured to the nearest 0.1 cm by a stadiometer (Holtain Limited, Crosswell, Crymych, UK). Weight was measured to the nearest gram using an electronic scale (SECA 888, Almere, the Netherlands). Body mass index (kg/m^2) was calculated.[25]

Total body and regional fat mass was measured using a dual energy x-ray absorptiometry (DXA) scanner (iDXA, 2008; GE-Lunar) and analyzed with the enCORE

software, version 12.6 (GE-Healthcare).[26] DXA can accurately detect whole body fat mass and regional body fat mass and has been validated against CT.[26,27] Total fat mass (kilograms) was calculated as a percentage of total body weight (kilograms) measured by DXA. The android/gynoid fat mass ratio was calculated. The android/gynoid fat ratio reflects the central body fat distribution in the abdomen and hip regions, respectively, and was used as a marker of waist/hip fat distribution.[27]

Abdominal ultrasound examinations were used to measure pre-peritoneal fat area as measure of visceral abdominal fat, as previously described.[28] Briefly, pre-peritoneal fat thickness was measured with a linear (L12-5 MHz) transducer[29], which was placed perpendicular to the skin surface on the median upper abdomen. We scanned longitudinally from the xiphoid process to the navel along the midline (linea alba). Preperitoneal fat mass area was measured as area of 2 cm length along the midline starting from the reference point in direction of the navel.

Covariates

Information on maternal age, educational level and ethnicity, was obtained at enrolment.[16] We measured maternal height and blood pressure at enrolment, and obtained information about maternal prepregnancy weight by questionnaire. We calculated body mass index. Information on maternal smoking, alcohol consumption and folic acid supplement use was assessed by questionnaires during pregnancy. Weight gain up to 30 weeks of gestation was calculated as the difference between maternal weight measured at 30 weeks of gestation (95% range, 28.4 - 32.9) and self-reported weight before pregnancy. As previously described, we used gestational weight gain until 30 weeks, because this was measured and available for 3,895 mothers.[30] Information about maximum weight during pregnancy was assessed by questionnaire 2 months after delivery and only available in a subgroup of 2,181 mothers. Maximum weight from questionnaire and weight measured at 30 weeks were strongly correlated ($r = 0.87$, $P < 0.001$). We used food frequency questionnaires to assess maternal nutritional information during pregnancy. Information about pregnancy complications, sex, gestational age and weight at birth was obtained from medical records.[31,32] Information about breastfeeding, timing of introduction of solid foods and average TV watching time was obtained by questionnaires in infancy.[33] Information about infant PUFA intake at 13 months, measured with a 211-item food frequency questionnaire was available in a subgroup of the study (n = 2,313).[34]

Statistical analysis

We explored the continuous associations of maternal PUFA concentrations with childhood adiposity outcomes at the age of 6 years using linear regression models. Because the abdominal

preperitoneal fat area was not normally distributed, we log transformed this variable for additional analyses. To enable comparison of effect estimates, we constructed standard deviation scores (SDS) ((observed value – mean) / SD) for all childhood adiposity outcomes and for levels of all PUFAs. We constructed different models; (1) a basic model including gestational age at maternal blood sampling, child's age and sex; (2) a pregnancy factor adjusted model, which was the basic model additionally adjusted for maternal age, educational level, ethnicity, parity, prepregnancy body mass index, gestational weight gain up to 30 weeks of gestation, blood pressure at enrolment, smoking, folic acid supplement use and total caloric intake during pregnancy and pregnancy complications; (3) a childhood factor adjusted model, which is the basic model additionally adjusted for gestational age and weight at birth, breastfeeding, timing of introduction of solid foods and average TV watching time; (4) and a fully adjusted model which includes all the above factors. All fat mass outcomes were additionally adjusted for child height. Included covariates were selected based on their associations with the outcomes of interest based on previous studies,[4,5] or a change in effect estimate of >10%. We performed an additional analysis by additionally adjusting these analyses for intake of PUFAs in infants at 13 months of age in a subgroup. We tested for interaction terms between maternal PUFA levels and child's sex in relation to adiposity outcomes in childhood. Since no statistically significant interactions were observed, no further stratified analyses were performed. In order to reduce potential bias associated with missing data and to maintain statistical power, we performed multiple imputations of missing covariates by generating 5 independent datasets using the Markov Chain Monte Carlo method after which the pooled effect estimates were calculated. All analyses were performed using Statistical Package for the Social Sciences version 21.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Subject characteristics

Table 3.2.1 shows the maternal and childhood characteristics. Mean (SD) second trimester maternal total n-3 and n-6 PUFA concentrations were 105.0 mg/L (27.5) and 604.2 mg/L (88.6), respectively **(Table 3.2.2)** Non-response analyses showed

that as compared to children with follow-up measurements, those without follow-up measurements had a lower gestational age at birth and lower birth weight (**Supplemental Table S3.2.1**). Also, mothers included in the analyses had a higher n-3 PUFA level and lower n-6/n-3 PUFAs ratio compared to those not included (**Supplemental Table S3.2.2**). Correlation coefficients between all maternal PUFA levels and childhood body fat outcomes are shown in **Supplemental Table 3.2.3.**

Table 3.2.1. Characteristics of mothers and their children (N =4,830)[1]

	Value
Maternal characteristics	
Age, median (y)	30.9 (19.9, 39.3)
Gestational age at PUFAs measures (weeks)	20.5 (16.5, 24.9)
Prepregnancy body mass index (kg/m^2)	23.6 ± 4.2
Weight gain up to 30 weeks of gestation (kg)	10.4 ± 4.9
Education, No. higher education (%)	2400 (46.2)
Ethnicity, No. European (%)	2917 (61.5)
Parity, No. nulliparous (%)	2757 (57.5)
Total energy intake (kcal)	2048 ± 558
Smoking during pregnancy No. Yes (%)	738 (17.1)
Folic acid supplements use, No. Yes (%)	2794 (75.6)
Preeclampsia, No. (%)	91 (2.0)
Gestational hypertension, No. (%)	182 (4.0)
Gestational diabetes, No. (%)	44 (0.9)
Birth and infant characteristics	
Males, No. (%)	2413 (50.0)
Gestational age at birth (weeks)	40.1 (35.8, 42.3)
Birth weight (g)	3433 ± 553
Ever breastfeeding, Yes, No. (%)	3550 (92.7)
Introduction of solid foods N. (%) >6 months	315 (10.4)
n-3 PUFAs intake (g/d)	0.6 (0.4)
n-6 PUFAs intake (g/d)	4.7 (2.9)
Childhood adiposity characteristics	
Age at follow up (y)	6.0 (5.6, 7.9)
Body mass index (kg/m^2)	16.2 ± 1.9
Fat mass percentage (%)	24.9 ± 5.7
Android / gynoid fat mass ratio	0.3 ± 0.1
Abdominal pre-peritoneal fat mass (cm^2)	0.39 (0.16, 1.2)

[1]Values represent means ± SDs, median (95% range) or number of subjects (valid%).
Abbreviations: PUFA: Polyunsaturated fatty acids.

Table 3.2.2. Second trimester maternal PUFA concentrations (N =4,830)[1]

	Absolute value (mg/L)	Percentage by weight of total sum of fatty acids (%)
Total PUFA concentrations	709.3 ± 97.9	43.1 ± 2.0
Total n-3 PUFA	105.0 ± 27.5	6.4 ± 1.5
ALA	5.1 ± 1.9	0.3 ± 0.1
EPA	8.7 ± 5.4	0.5 ± 0.3
DPA	12.1 ± 4.3	0.7 ± 0.2
DHA	77.6 ± 20.4	4.8 ± 1.1
Total n-6 PUFA	604.2 ± 88.6	36.8 ± 2.5
LA	361.9 ± 63.1	22.3 ± 2.8
GLA	1.5 ± 0.7	0.1 ± 0.0
EDA	8.5 ± 1.9	0.5 ± 0.1
DGLA	61.0 ± 16.5	3.7 ± 0.7
AA	156.4 ± 32.6	9.6 ± 1.6
DTA	6.9 ± 2.2	0.4 ± 1.1
Total n-6/n-3 PUFAs ratio	6.1 ± 1.7	-

[1]Values represent means ± SDs.

Abbreviations: AA: arachidonic acid; ALA: α-linolenic acid; DGLA dihomo-γ-linolenic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; DTA: docosatetraenoic acid; EDA: eicosadienoic acid; EPA: eicosapentaenoic acid; GLA: γ-linolenic acid; LA: linoleic acid; PUFA; polyunsaturated fatty acid;

Maternal n-3 PUFA concentrations and childhood body fat outcomes

Table 3.2.3 shows that in the basic models, higher maternal total n-3 PUFA concentrations and concentrations of each n-3 fatty acid individually were associated with lower childhood body mass index, total body fat mass percentage, android/gynoid fat mass ratio and abdominal preperitoneal fat mass area (all *P* values <0.05). In the fully adjusted models, all n-3 PUFA concentrations except for ALA were still associated with lower childhood total body fat mass percentage and android/gynoid fat mass ratio (all *P* values <0.05), but not with childhood body mass index and preperitoneal fat mass area. The strongest effect estimate was observed for the associations of maternal total n-3 PUFA concentrations with childhood total body fat mass percentage (difference: -0.07 (95% CI: -0.10, -0.05) per SD increase of total maternal n-3 PUFA in the fully adjusted model). Models adjusted for pregnancy and childhood factors separately are given in **Supplemental Table S3.2.4** and show that pregnancy factors fully explained the associations of all maternal total n-3 PUFAs with childhood body mass index and abdominal preperitoneal fat mass area. Childhood factors did not materially influence the observed associations.

Table 3.2.3. Maternal n-3 PUFA concentrations and childhood body fat outcomes (N =4,830)[1]

Maternal n-3 PUFAs in SDS	Differences in childhood adiposity outcomes (95% confidence interval) in SDS			
	Body mass index (N=4,830)	Total body fat mass percentage (N=4,706)	Android/gynoid fat mass ratio (N=4,706)	Preperitoneal fat mass area (N=3,912)
Total n-3 PUFAs				
Basic model[2]	-0.09 (-0.12, -0.07)*	-0.16 (-0.18, -0.13)*	-0.13 (-0.15, -0.10)*	-0.09 (-0.12, -0.06)*
Full model[3]	-0.02 (-0.05, 0.01)	-0.07 (-0.10, -0.05)*	-0.07 (-0.10, -0.04)*	-0.02 (-0.06, 0.01)
ALA				
Basic model	-0.08 (-0.11, -0.05)*	-0.10 (-0.12, -0.07)*	-0.07 (-0.10, -0.04)*	-0.08 (-0.11, -0.06)*
Full model	0.00 (-0.03, 0.03)	-0.02 (-0.05, 0.00)	-0.02 (-0.05, 0.01)	-0.03 (-0.06, -0.00)*
EPA				
Basic model	-0.08 (-0.10, -0.05)*	-0.13 (-0.15, -0.10)*	-0.09 (-0.12, -0.07)*	-0.07 (-0.10, -0.05)*
Full model	-0.02 (-0.04, 0.01)	-0.06 (-0.08, -0.03)*	-0.04 (-0.07, -0.01)*	-0.02 (-0.05, 0.01)
DPA				
Basic model	-0.05 (-0.08, -0.03)*	-0.11 (-0.14, -0.09)*	-0.08 (-0.11, -0.06)*	-0.04 (-0.07, -0.01)*
Full model	-0.00 (-0.03, 0.03)	-0.05 (-0.08, -0.03)*	-0.05 (-0.08, -0.02)*	0.00 (-0.03, 0.03)
DHA				
Basic model	-0.08 (-0.11, -0.05)*	-0.13 (-0.16, -0.11)*	-0.11 (-0.13, -0.08)*	-0.07 (-0.10, -0.05)*
Full model	-0.02 (-0.04, 0.01)	-0.07 (-0.09, -0.04)*	-0.06 (-0.09, -0.03)*	-0.02 (-0.05, 0.01)

[1]Values are regression coefficients (95% CIs) that reflect the difference in SDS of childhood body mass index, total body fat mass percentage, android/gynoid fat mass ratio and abdominal preperitoneal fat mass area per SD change in maternal n-3 PUFA concentrations, respectively. [2]Basic models are adjusted for gestational age at blood sampling, child age, sex and height (fat mass outcomes only). [3]Full models are adjusted for pregnancy factors and childhood factors, which included: maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, weight gain up to 30 weeks of gestation, blood pressure at enrolment, and smoking, folic acid supplement use and total calorie intake during pregnancy and pregnancy complications, gestational age and weight at birth, breastfeeding duration, timing of introduction of solid foods and TV watching time, respectively. * *P*-value<0.05.
Abbreviations: ALA: α-linolenic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; PUFA; polyunsaturated fatty acid; SD: standard deviation.

Maternal n-6 PUFA concentrations and childhood body fat outcomes

Table 3.2.4 shows that in the basic models, higher maternal total n-6 PUFA concentrations, LA, DGLA and DTA concentrations, were associated with higher childhood body mass index, total body fat mass percentage, android/gynoid fat mass ratio and abdominal preperitoneal fat mass area (all *P* values <0.05). After adjustment for maternal and childhood characteristics, higher total maternal n-6 PUFA concentrations and DGLA concentrations were associated with a higher childhood total body fat mass percentage, android/gynoid fat mass ratio and abdominal preperitoneal fat mass area (all *P* values<0.05), but not with childhood body mass index. The strongest effect estimate was observed for the associations of maternal total n-6 PUFA concentrations with childhood total body fat mass percentage (difference: 0.08 (95% CI: 0.05, 0.10) per SD increase of total maternal n-6 PUFA in the fully adjusted model). Models adjusted for pregnancy and childhood factors separately are given in **Supplemental Table S3.2.5** and show that pregnancy factors fully explained the associations of maternal total n-6 PUFAs with childhood body mass index.

Table 3.2.4. Maternal n-6 PUFA concentrations and childhood body fat outcomes (N =4,830)[1]

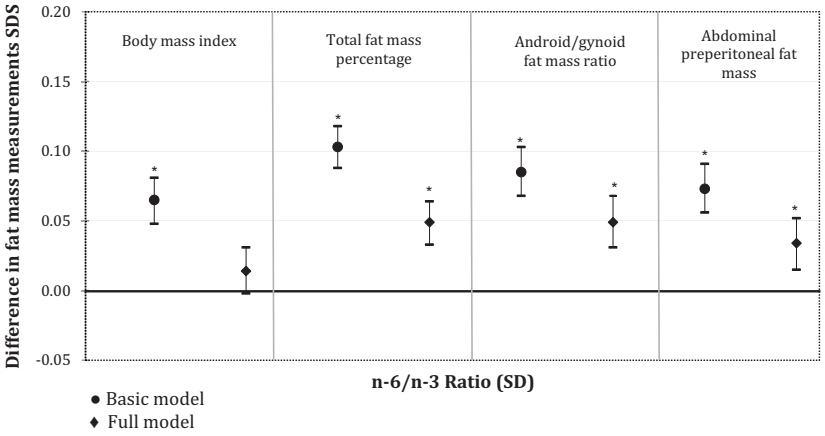
	Differences in childhood adiposity outcomes (95% confidence interval) in SDS			
Maternal n-6 PUFAs in SDS	**Body mass index (N=4,830)**	**Total body fat mass percentage (N=4,706)**	**Android/gynoid fat mass ratio (N=4,706)**	**Preperitoneal fat mass area (N=3,912)**
Total n-6 PUFA				
Basic model[2]	0.11 (0.08, 0.14)*	0.17 (0.15, 0.20)*	0.12 (0.09, 0.15)*	0.14 (0.11, 0.17)*
Full model[3]	0.03 (-0.00, 0.06)	0.08 (0.05, 0.10)*	0.06 (0.03, 0.10)*	0.07 (0.04, 0.11)*
LA				
Basic model	0.03 (0.00, 0.06)*	0.08 (0.06, 0.11)*	0.07 (0.04, 0.10)*	0.06 (0.03, 0.08)*
Full model	0.01 (-0.02, 0.04)	0.05 (0.03, 0.08)*	0.06 (0.03, 0.09)*	0.03 (-0.00, 0.06)
GLA				
Basic model	0.03 (-0.00, 0.05)	0.03 (0.01, 0.06)*	0.02 (-0.01, 0.05)	0.06 (0.03, 0.08)*
Full model	0.01 (-0.02, 0.03)	0.01 (-0.01, 0.04)	-0.00 (-0.03, 0.03)	0.04 (0.01, 0.07)*
EDA				
Basic model	-0.00 (-0.03, 0.03)	0.02 (-0.01, 0.05)	0.02 (-0.01, 0.05)	0.01 (-0.02, 0.04)
Full model	-0.01 (-0.03, 0.02)	0.00 (-0.02, 0.03)	0.02 (-0.01, 0.05)	-0.01 (-0.04, 0.02)
DGLA				
Basic model	0.06 (0.03, 0.09)*	0.06 (0.04, 0.09)*	0.07 (0.04, 0.09)*	0.07 (0.04, 0.10)*
Full model	0.01 (-0.02, 0.03)	0.04 (0.02, 0.07)*	0.05 (0.02, 0.08)*	0.06 (0.03, 0.09)*
AA				
Basic model	0.09 (0.06, 0.11)*	0.08 (0.05, 0.10)*	0.03 (-0.00, 0.05)	0.08 (0.05, 0.11)*
Full model	0.01 (-0.02, 0.04)	-0.01 (-0.04, 0.02)	-0.04 (-0.07, -0.01)*	0.02 (-0.01, 0.05)
DTA				
Basic model	0.08 (0.06, 0.11)*	0.08 (0.06, 0.11)*	0.06 (0.03, 0.09)*	0.10 (0.07, 0.12)*
Full model	0.02 (-0.01, 0.05)	0.01 (-0.02, 0.04)	0.01 (-0.02, 0.04)	0.04 (0.01, 0.07)*

[1]Values are regression coefficients (95% CIs) that reflect the difference in SDS of childhood body mass index, total body fat mass percentage, android/gynoid fat mass ratio and abdominal preperitoneal fat mass area per SD change in maternal n-6 PUFA concentrations, respectively. [2]Basic models are adjusted for gestational age at blood sampling, child age, sex and height (fat mass outcomes only).[3]Full models are adjusted for pregnancy factors and childhood factors, which included: maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, weight gain up to 30 weeks of gestation, blood pressure at enrolment, and smoking, folic acid supplement use and total calorie intake during pregnancy and pregnancy complications, gestational age and weight at birth, breastfeeding duration, timing of introduction of solid foods and TV watching time, respectively. * *P*-value<0.05.
Abbreviations: AA: arachidonic acid; DGLA dihomo-γ-linolenic acid; DTA: docosatetraenoic acid; GLA: γ-linolenic acid, EDA: eicosadienoic acid; LA: linoleic acid; PUFA; polyunsaturated fatty acid; SD: standard deviation.

Maternal n-6/n-3 PUFAs ratio and childhood body fat outcomes

Figure 3.2.1 shows that a higher maternal n-6/n-3 PUFA ratio was associated with a higher childhood body mass index, total body fat mass percentage, android/gynoid fat mass ratio and abdominal preperitoneal fat mass area in the basic model (all *P* values<0.05). In the fully adjusted model, a higher maternal n-6/n-3 PUFAs ratio was associated with a higher childhood total body fat mass percentage, android/gynoid fat mass ratio and abdominal preperitoneal fat mass area (differences: 0.05 (95% CI: 0.03, 0.06), 0.05 (95% CI: 0.03, 0.07) and 0.03 (95% CI: 0.02, 0.05) per SD increase in total n-6/n-3 PUFAs ratio, respectively). Associations of maternal n-6/n-3 PUFAs ratio with childhood body fat outcomes adjusted for pregnancy and childhood factors are presented in **Supplemental Figure S3.2.2.**

Figure 3.2.1 Maternal n-6/n-3 PUFAs ratio and childhood body fat outcomes (N =4,830)



Values are regression coefficients (95% CIs) that reflect the difference in SDS of childhood body mass index, total body fat mass percentage, android/gynoid fat mass ratio and abdominal pre-peritoneal fat mass area per SD change in maternal n-6/n-3 PUFAs ratio, respectively. Basic models are adjusted for gestational age at blood sampling, child age, sex and height (fat mass outcomes only). Full models are adjusted for pregnancy factors and childhood factors, which included: maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, weight gain up to 30 weeks of gestation, blood pressure at enrolment, and smoking, folic acid supplement use and total calorie intake during pregnancy and pregnancy complications, gestational age and weight at birth, breastfeeding duration, timing of introduction of solid foods and TV watching time, respectively. * P-value<0.05.
Abbreviations: PUFA; polyunsaturated fatty acid; SD: standard deviation.

Additional analysis to take infant PUFA intake into account

We performed an additional analysis among 2,313 children to explore whether the associations were explained by infant PUFA intake. We observed that additional adjustment for PUFA intake in infants did not materially affect the observed associations (data not shown).

Discussion

In this population based prospective cohort study, we observed that maternal lower n-3 PUFA concentrations and higher n-6 PUFA concentrations during pregnancy are associated with a higher body fat percentage and an adverse general and abdominal fat distribution in childhood. The associations of maternal n-3 and n-6 PUFA concentrations with detailed childhood fat mass outcomes were only partly explained by maternal and childhood characteristics.

Methodological considerations

We used a population-based prospective cohort study design with a large number of subjects. Of all children whose maternal PUFAconcentrations were available, 64% participated in the follow-up studies at the age of 6 years. The non-response could lead to biased effect estimates if the associations of maternal PUFA concentrations with childhood body composition would be different between children included and not included in the analyses. Non-response analysis showed that birth weight and gestational age at birth were lower among children who were not included in the analyses compared to those who were included. Also, the n-6/n-3 PUFA ratio was higher among mothers of children who were not included in the analysis. Selective loss to follow up may have led to underestimation of the effect estimates if children with adverse body fat profiles from mothers with higher n-6/n-3 ratios had a higher risk of loss to follow-up. We measured a large number of maternal PUFA concentrations in blood samples only once during pregnancy. These concentrations of maternal PUFAs may not fully reflect the concentrationsof PUFA that the fetus was exposed to during the full pregnancy, as this also depends on placental transfer.[35] Also, PUFAs measured in plasma may reflect a time frame of dietary intake of approximately 2 weeks and seem to be reasonable indicators for the recent intake.[36] Unfortunately, no information was available about erythrocyte lipid levels, which reflect a longer intake period. We performed detailed measurements of childhood body fat distribution outcomes. DXA quantifies the fat content with a high precision and has the capacity for a regional analysis but cannot differentiate the two abdominal fat compartments.[26,27] Ultrasound is a reliable method to differentiate between abdominal visceral and subcutaneous fat compartments by using an area measurement as a proxy for these fat compartments.[37] Both DXA and abdominal ultrasound have been validated against computed tomography.[26,27,37] We presented the associations of plasma PUFA concentrations with childhood adiposity outcomes in different regression models (basic model; pregnancy model; childhood model; fully adjusted model) to enable interpretation of the results with and without different adjustment approaches. We observed the largest changes in effect estimates when we additionally adjusted the basic model for maternal prepregnancy body mass index. Further adjustment for childhood factors did not change the effect estimates. We included various possible confounders because of the potential of residual confounding in this observational study. However, we may have overadjusted the fully adjusted models by including various covariates potentially involved in causal pathways. Despite extensive adjustment, residual confounding may still be an issue. Most importantly, we did not have detailed information about childhood PUFA blood concentrations or dietary intake available in the full cohort. However, one previous study found that adjustment for

child PUFA concentrations did not change the association between maternal PUFA concentrations and childhood body composition.[5] Further studies are needed to further explore the potential role of confounding by maternal and childhood dietary factors in these observed associations.

Interpretation of main findings

An adequate PUFA supply is important for optimal fetal development.[38] Previous studies suggested that lower maternal n-3 PUFA and higher n-6 PUFA concentrations are associated with an increased risk of adverse birth outcomes.[4,21] Alterations in maternal n-3 and n-6 PUFA concentrations might also have long-term offspring consequences.[35] Several studies suggested that lower maternal n-3 PUFA concentrations and higher n-6 PUFA concentrations are associated with a higher body mass index in the offspring, but results are not consistent.[5,15,39,40] A study among 234 mother-child pairs of the Maastricht Essential Fatty Acid Birth (MEFAB) cohort in the Netherlands showed that higher maternal DGLA, an n-6 PUFA, during pregnancy was associated with increased childhood body mass index at age 7.[5] In another study among 388 German mother-child pairs a higher n-6/n-3 PUFAs ratio in cord blood was associated with a higher body mass index at 10 years of life.[41] Another study among 208 pregnant women in Germany showed that higher concentrations of maternal AA, an n-6 PUFA, were associated with lower offspring body mass index at 1 year of age.[42] Thus, results from previous observational studies suggest that maternal PUFA concentrations during pregnancy have a persistent effect on childhood growth and adiposity. Such observational studies may suffer from residual confounding. To take confounding into account in the associations of maternal PUFA concentrations and childhood adiposity outcomes, several randomized trials have been performed. A randomized, double-blind clinical trial among 144 pregnant women showed that infants of mothers who received 200 mg DHA, an n-3 PUFA, per day during pregnancy had a lower body mass index at 21 months, but there was no difference at age 6 years.[43] A meta-analysis of 6 randomized controlled trials showed no effect of maternal n-3 PUFAs supplementation during pregnancy on body mass index in preschool children.[14] Thus results from these trials do not support the results obtained in observational studies. However, due to the subject selection and specific composition of the supplements, results from these trials are difficult to generalize to larger population-based samples.

Only a few studies assessed the associations of maternal PUFA concentrations with more detailed offspring body fat measures. A study among 1,250 mother-child pairs in Massachusetts showed that higher concentrations of DHA and EPA, n-3 PUFAs, in the maternal diet and in umbilical cord, were associated with lower subcutaneous

fat mass measured by the sum of subscapular and triceps skinfold thicknesses at the age of 3 years.[21] In the same study, higher maternal concentration of n-6 PUFAs were associated with higher sum of childhood subscapular and triceps skinfold thicknesses.[21] In this study, a food frequency questionnaire was used to assess maternal fatty acid intake. A prospective UK cohort study among 293 mother-child pairs showed that higher maternal n-6 PUFAs concentrations were positively associated with offspring body fat mass measured by DXA at 6 years. The maternal n-3/n-6 PUFAs ratio was negatively associated with offspring fat mass at 4 years, but not at 6 years.[4] Findings from this latter study suggest that a reduction in maternal n-6 PUFAs concentrations is more protective than increasing the concentration of n-3 PUFAs for children. A randomized trial among 208 pregnant women showed no effect of n-3 PUFAs supplementation on fat mass, as assessed by skinfold thickness and abdominal ultrasonography, in the offspring during the first year of life.[12]

In the current study, we observed in the basic models that maternal higher n-3 PUFAs and lower total n-6 PUFAs were associated with a lower body mass index in childhood. However, these associations were fully explained by maternal sociodemographic and lifestyle-related characteristics. Especially, maternal body mass index seems to largely explain the associations of maternal PUFA concentrations and childhood body mass index. In contrast to the associations with childhood body mass index, we observed that higher maternal total n-3 PUFA concentrations were associated with lower childhood total body fat mass, android/gynoid fat mass ratio, a measure of waist-to-hip ratio and abdominal pre-peritoneal fat mass area, a marker of visceral fat mass. Concentrations of the n-3-PUFAs, ALA, EPA, DPA, and DHA were all associated with these childhood outcomes. Higher maternal total n-6 PUFA concentrations, specifically LA, DGLA, AA, and DTA were associated with higher childhood total body fat mass, android/gynoid fat mass ratio, and abdominal pre-peritoneal fat mass area. In line with these findings, a higher maternal n-6/n-3 PUFAs ratio was also associated with higher childhood total body and abdominal fat mass levels. These associations were not explained by the maternal and childhood factors on which we had information available. Thus, our results suggest that both lower maternal n-3 PUFA concentrations and higher maternal n-6 PUFA concentrations during pregnancy affect childhood total body fat mass and abdominal fat mass levels. An adverse body fat distribution seems to be, independently of body mass index, associated with an adverse cardiovascular risk profile.[10,44,45] Whether the observed body fat differences related to maternal PUFA concentrations have adverse health consequences should be further studied.

Because of the observational design of the study, further studies are needed for replication and to explore causality. If the associations are indeed causal, the biologi-

cal mechanisms underlying may involve adaptations in fetal fat cell development and adipose tissue growth.[4,46] N-6 PUFAs forms a precursor of prostacyclin, which promotes the differentiation of preadipocytes into mature and functional adipocytes.[47] On the contrary, n-3 PUFAs inhibit this process of differentiation through the inhibition of the activity of the cyclooxygenase enzymes, which enhance prostaglandin biosynthesis.[48] Thus, n-3 PUFAs inhibit the process of lipid storage and accumulation in the fetuses reducing both hyperplasia as well as hypertrophy of growing fat depots.[48] Our results are important from an etiological perspective. Although the observed effect estimates were small and without clinical relevance for individuals, the results may be relevant on population levels. Unfortunately, causality cannot be established by this observational study. Experimental and Mendelian Randomization studies may help to assess the causality of the observed associations.[49]

Conclusion

We observed that higher maternal n-3 PUFA concentrations and lower maternal n-6 PUFA concentrations during pregnancy are associated with a lower childhood total body fat and abdominal fat levels. These associations were only partly explained by maternal and childhood socio-demographic and lifestyle-related characteristics. Further observational and experimental studies are needed for replication and to explore the causality and long-term cardio-metabolic consequences.

References

1. Baird J, Fisher D, Lucas P, Kleijnen J, Roberts H, Law C. Being big or growing fast: systematic review of size and growth in infancy and later obesity. BMJ 2005;331(7522):929.
2. Cetin I, Alvino G, Cardellicchio M. Long chain fatty acids and dietary fats in fetal nutrition. J Physiol 2009;587(Pt 14):3441-3451.
3. Muhlhausler BS, Gibson RA, Makrides M. The effect of maternal omega-3 long-chain polyunsaturated fatty acid (n-3 LCPUFA) supplementation during pregnancy and/or lactation on body fat mass in the offspring: a systematic review of animal studies. Prostaglandins Leukot Essent Fatty Acids 2011;85(2):83-88.
4. Moon RJ, Harvey NC, Robinson SM, Ntani G, Davies JH, Inskip HM, Godfrey KM, Dennison EM, Calder PC, Cooper C, Group SWSS. Maternal plasma polyunsaturated fatty acid status in late pregnancy is associated with offspring body composition in childhood. J Clin Endocrinol Metab 2013;98(1):299-307.
5. de Vries PS, Gielen M, Rizopoulos D, Rump P, Godschalk R, Hornstra G, Zeegers MP. Association between polyunsaturated fatty acid concentrations in maternal plasma phospholipids during preg-

nancy and offspring adiposity at age 7: the MEFAB cohort. Prostaglandins Leukot Essent Fatty Acids 2014;91(3):81-85.
6. Shah NR, Braverman ER. Measuring adiposity in patients: the utility of body mass index (BMI), percent body fat, and leptin. PLoS One 2012;7(4):e33308.
7. Savva SC, Tornaritis M, Savva ME, Kourides Y, Panagi A, Silikiotou N, Georgiou C, Kafatos A. Waist circumference and waist-to-height ratio are better predictors of cardiovascular disease risk factors in children than body mass index. Int J Obes Relat Metab Disord 2000;24(11):1453-1458.
8. Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, Eckel RH, American Heart A, Obesity Committee of the Council on Nutrition PA, Metabolism. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. Circulation 2006;113(6):898-918.
9. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, Allen K, Lopes M, Savoye M, Morrison J, Sherwin RS, Caprio S. Obesity and the metabolic syndrome in children and adolescents. N Engl J Med 2004;350(23):2362-2374.
10. Gishti O, Gaillard R, Durmus B, Abrahamse M, van der Beek EM, Hofman A, Franco OH, de Jonge LL, Jaddoe VWV. Body mass index, total and abdominal fat distribution and cardiovascular risk factors in school-age children. 2015.
11. Helland IB, Smith L, Blomen B, Saarem K, Saugstad OD, Drevon CA. Effect of supplementing pregnant and lactating mothers with n-3 very-long-chain fatty acids on children's IQ and body mass index at 7 years of age. Pediatrics 2008;122(2):e472-479.
12. Hauner H, Much D, Vollhardt C, Brunner S, Schmid D, Sedlmeier EM, Heimberg E, Schuster T, Zimmermann A, Schneider KT, Bader BL, Amann-Gassner U. Effect of reducing the n-6:n-3 long-chain PUFA ratio during pregnancy and lactation on infant adipose tissue growth within the first year of life: an open-label randomized controlled trial. Am J Clin Nutr 2012;95(2):383-394.
13. Rytter D, Bech BH, Christensen JH, Schmidt EB, Henriksen TB, Olsen SF. Intake of fish oil during pregnancy and adiposity in 19-y-old offspring: follow-up on a randomized controlled trial. Am J Clin Nutr 2011;94(3):701-708.
14. Stratakis N, Gielen M, Chatzi L, Zeegers MP. Effect of maternal n-3 long-chain polyunsaturated fatty acid supplementation during pregnancy and/or lactation on adiposity in childhood: a systematic review and meta-analysis of randomized controlled trials. Eur J Clin Nutr 2014;68(12):1277-1287.
15. Rodriguez G, Iglesia I, Bel-Serrat S, Moreno LA. Effect of n-3 long chain polyunsaturated fatty acids during the perinatal period on later body composition. Br J Nutr 2012;107 Suppl 2:S117-128.
16. Jaddoe VW, van Duijn CM, Franco OH, van der Heijden AJ, van Iizendoorn MH, de Jongste JC, van der Lugt A, Mackenbach JP, Moll HA, Raat H, Rivadeneira F, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC, Hofman A. The Generation R Study: design and cohort update 2012. Eur J Epidemiol 2012;27(9):739-756.
17. Kruithof CJ, Kooijman MN, van Duijn CM, Franco OH, de Jongste JC, Klaver CC, Mackenbach JP, Moll HA, Raat H, Rings EH, Rivadeneira F, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC, Wolvius EB, Hofman A, Jaddoe VW. The Generation R Study: Biobank update 2015. Eur J Epidemiol 2014;29(12):911-927.
18. Glaser C, Demmelmair H, Koletzko B. High-throughput analysis of fatty acid composition of plasma glycerophospholipids. J Lipid Res 2010;51(1):216-221.

19. Jaddoe VW, Bakker R, van Duijn CM, van der Heijden AJ, Lindemans J, Mackenbach JP, Moll HA, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC, Hofman A. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. Eur J Epidemiol 2007;22(12):917-923.
20. Steenweg-de Graaff JC, Tiemeier H, Basten MG, Rijlaarsdam J, Demmelmair H, Koletzko B, Hofman A, Jaddoe VW, Verhulst FC, Roza SJ. Maternal LC-PUFA status during pregnancy and child problem behavior: the Generation R Study. Pediatr Res 2015;77(3):489-497.
21. Donahue SM, Rifas-Shiman SL, Gold DR, Jouni ZE, Gillman MW, Oken E. Prenatal fatty acid status and child adiposity at age 3 y: results from a US pregnancy cohort. Am J Clin Nutr 2011;93(4):780-788.
22. Koletzko B, Lien E, Agostoni C, Bohles H, Campoy C, Cetin I, Decsi T, Dudenhausen JW, Dupont C, Forsyth S, Hoesli I, Holzgreve W, Lapillonne A, Putet G, Secher NJ, Symonds M, Szajewska H, Willatts P, Uauy R, World Association of Perinatal Medicine Dietary Guidelines Working G. The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. J Perinat Med 2008;36(1):5-14.
23. Kinsella JE, Lokesh B, Stone RA. Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. Am J Clin Nutr 1990;52(1):1-28.
24. de Groot RH, Hornstra G, van Houwelingen AC, Roumen F. Effect of alpha-linolenic acid supplementation during pregnancy on maternal and neonatal polyunsaturated fatty acid status and pregnancy outcome. Am J Clin Nutr 2004;79(2):251-260.
25. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ 2000;320(7244):1240-1243.
26. Kaul S, Rothney MP, Peters DM, Wacker WK, Davis CE, Shapiro MD, Ergun DL. Dual-energy X-ray absorptiometry for quantification of visceral fat. Obesity (Silver Spring) 2012;20(6):1313-1318.
27. Helba M, Binkovitz LA. Pediatric body composition analysis with dual-energy X-ray absorptiometry. Pediatr Radiol 2009;39(7):647-656.
28. Mook-Kanamori DO, Holzhauer S, Hollestein LM, Durmus B, Manniesing R, Koek M, Boehm G, van der Beek EM, Hofman A, Witteman JC, Lequin MH, Jaddoe VW. Abdominal fat in children measured by ultrasound and computed tomography. Ultrasound Med Biol 2009;35(12):1938-1946.
29. Suzuki R, Watanabe S, Hirai Y, Akiyama K, Nishide T, Matsushima Y, Murayama H, Ohshima H, Shinomiya M, Shirai K, et al. Abdominal wall fat index, estimated by ultrasonography, for assessment of the ratio of visceral fat to subcutaneous fat in the abdomen. Am J Med 1993;95(3):309-314.
30. Gaillard R, Durmus B, Hofman A, Mackenbach JP, Steegers EA, Jaddoe VW. Risk factors and outcomes of maternal obesity and excessive weight gain during pregnancy. Obesity (Silver Spring) 2013;21(5):1046-1055.
31. Verburg BO, Steegers EA, De Ridder M, Snijders RJ, Smith E, Hofman A, Moll HA, Jaddoe VW, Witteman JC. New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. Ultrasound Obstet Gynecol 2008;31(4):388-396.
32. Niklasson A, Ericson A, Fryer JG, Karlberg J, Lawrence C, Karlberg P. An update of the Swedish reference standards for weight, length and head circumference at birth for given gestational age (1977-1981). Acta Paediatr Scand 1991;80(8-9):756-762.
33. Heppe DH, Medina-Gomez C, Hofman A, Franco OH, Rivadeneira F, Jaddoe VW. Maternal first-trimester diet and childhood bone mass: the Generation R Study. Am J Clin Nutr 2013;98(1):224-232.

34. Voortman T, Kiefte-de Jong JC, Geelen A, Villamor E, Moll HA, de Jongste JC, Raat H, Hofman A, Jaddoe VW, Franco OH, van den Hooven EH. The development of a diet quality score for preschool children and its validation and determinants in the Generation R Study. J Nutr 2015;145(2):306-314.
35. Hornstra G. Essential fatty acids in mothers and their neonates. Am J Clin Nutr 2000;71(5 Suppl):1262S-1269S.
36. Skeaff CM, Hodson L, McKenzie JE. Dietary-induced changes in fatty acid composition of human plasma, platelet, and erythrocyte lipids follow a similar time course. J Nutr 2006;136(3):565-569.
37. Bazzocchi A, Filonzi G, Ponti F, Sassi C, Salizzoni E, Battista G, Canini R. Accuracy, reproducibility and repeatability of ultrasonography in the assessment of abdominal adiposity. Acad Radiol 2011;18(9):1133-1143.
38. Koletzko B, Boey CC, Campoy C, Carlson SE, Chang N, Guillermo-Tuazon MA, Joshi S, Prell C, Quak SH, Sjarif DR, Su Y, Supapannachart S, Yamashiro Y, Osendarp SJ. Current information and Asian perspectives on long-chain polyunsaturated fatty acids in pregnancy, lactation, and infancy: systematic review and practice recommendations from an early nutrition academy workshop. Ann Nutr Metab 2014;65(1):49-80.
39. Hauner H, Brunner S, Amann-Gassner U. The role of dietary fatty acids for early human adipose tissue growth. Am J Clin Nutr 2013;98(2):549S-555S.
40. Muhlhausler BS, Ailhaud GP. Omega-6 polyunsaturated fatty acids and the early origins of obesity. Curr Opin Endocrinol Diabetes Obes 2013;20(1):56-61.
41. Standl M, Thiering E, Demmelmair H, Koletzko B, Heinrich J. Age-dependent effects of cord blood long-chain PUFA composition on BMI during the first 10 years of life. Br J Nutr 2014:1-8.
42. Much D, Brunner S, Vollhardt C, Schmid D, Sedlmeier EM, Bruderl M, Heimberg E, Bartke N, Boehm G, Bader BL, Amann-Gassner U, Hauner H. Effect of dietary intervention to reduce the n-6/n-3 fatty acid ratio on maternal and fetal fatty acid profile and its relation to offspring growth and body composition at 1 year of age. Eur J Clin Nutr 2013;67(3):282-288.
43. Lucia Bergmann R, Bergmann KE, Haschke-Becher E, Richter R, Dudenhausen JW, Barclay D, Haschke F. Does maternal docosahexaenoic acid supplementation during pregnancy and lactation lower BMI in late infancy? J Perinat Med 2007;35(4):295-300.
44. Pischon T, Boeing H, Hoffmann K, Bergmann M, Schulze MB, Overvad K, van der Schouw YT, Spencer E, Moons KG, Tjonneland A, Halkjaer J, Jensen MK, Stegger J, Clavel-Chapelon F, Boutron-Ruault MC, Chajes V, Linseisen J, Kaaks R, Trichopoulou A, Trichopoulos D, Bamia C, Sieri S, Palli D, Tumino R, Vineis P, Panico S, Peeters PH, May AM, Bueno-de-Mesquita HB, van Duijnhoven FJ, Hallmans G, Weinehall L, Manjer J, Hedblad B, Lund E, Agudo A, Arriola L, Barricarte A, Navarro C, Martinez C, Quiros JR, Key T, Bingham S, Khaw KT, Boffetta P, Jenab M, Ferrari P, Riboli E. General and abdominal adiposity and risk of death in Europe. N Engl J Med 2008;359(20):2105-2120.
45. Srinivasan SR, Wang R, Chen W, Wei CY, Xu J, Berenson GS. Utility of waist-to-height ratio in detecting central obesity and related adverse cardiovascular risk profile among normal weight younger adults (from the Bogalusa Heart Study). Am J Cardiol 2009;104(5):721-724.
46. Ailhaud G, Massiera F, Weill P, Legrand P, Alessandri JM, Guesnet P. Temporal changes in dietary fats: role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity. Prog Lipid Res 2006;45(3):203-236.
47. Ailhaud G, Guesnet P, Cunnane SC. An emerging risk factor for obesity: does disequilibrium of polyunsaturated fatty acid metabolism contribute to excessive adipose tissue development? Br J Nutr 2008;100(3):461-470.

48. Ringbom T, Huss U, Stenholm A, Flock S, Skattebol L, Perera P, Bohlin L. Cox-2 inhibitory effects of naturally occurring and modified fatty acids. J Nat Prod 2001;64(6):745-749.
49. Smith GD, Ebrahim S. Mendelian randomization: prospects, potentials, and limitations. Int J Epidemiol 2004;33(1):30-42.

Supplemental Material

Figure S3.2.1. Flow chart of the participants

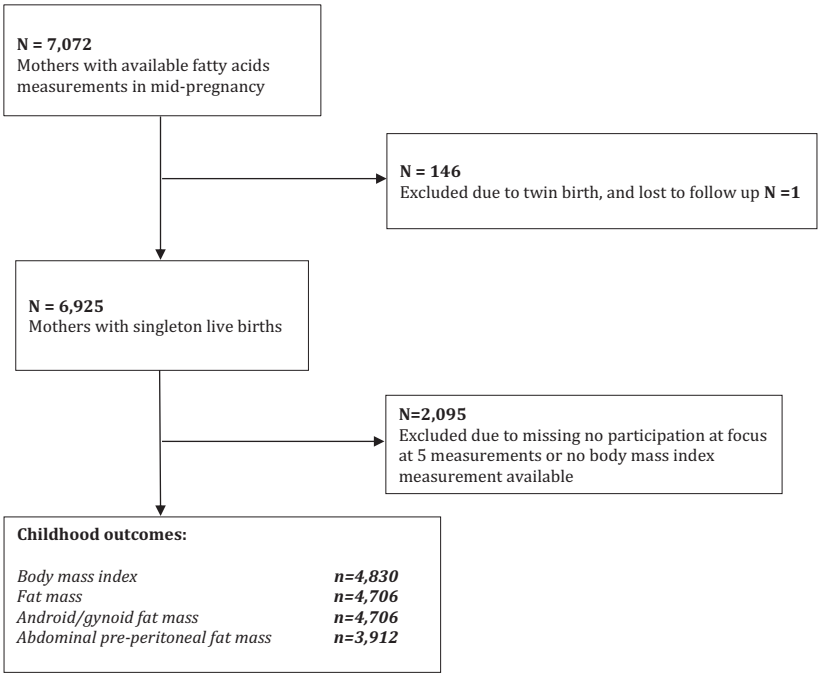


Table S3.2.1. Comparison of subject characteristics between those included and not included in the analyses (N = 6,924)[1]

Characteristics	Participation in follow-up N = 4,830	No participation in follow-up N = 2,094	P-value
Maternal Characteristics			
Age (y)	30.9 (19.9, 39.3)	28.8 (18.5, 38.7)	<0.01
Gestational age at PUFA measures (weeks)	20.5 (16.5, 24.9)	20.5 (17.1, 24.9)	0.99
Pre-pregnancy body mass index (kg/m^2)	23.6 ± 4.2	23.7 ± 4.7	0.48
Weight gain up to 30 weeks of gestation (kg)	10.4 ± 4.9	10.3 ± 5.4	0.79
Education, No. higher education (%)	2400 (46.2)	602 (33.0)	<0.01
Ethnicity, European No. (%)	2917 (61.5)	897 (48.0)	<0.01
Parity, No. nulliparous (%)	2757 (57.5)	1087 (52.7)	<0.01
Total energy intake, mean ± SD, kcal/d	2048 ± 558	2020 ± 591	0.13
Smoking during pregnancy No. Yes (%)	738 (17.1)	407 (22.5)	<0.01
Folic acid supplements use, No. Yes (%)	2794 (75.6)	911 (61.4)	<0.01
Preeclampsia	91 (2.0)	55 (2.9)	0.04
Gestational hypertension	182 (4.0)	58 (3.0)	0.06
Gestational diabetes	44 (0.9)	26 (1.3)	0.17
Childhood characteristics			
Males, No. (%)	2413 (50.0)	1086 (51.9)	0.14
Gestational age at birth, median (95% range), weeks	40.1 (35.8, 42.3)	40.0 (34.9, 42.4)	<0.01
Birth weight, mean ± SD, g	3433 ± 553	3383 ± 579	<0.01
Ever breastfeeding, Yes, No. (%)	3550 (92.7)	1014 (90.5)	0.02
Introduction of solid foods No. (%) >6 months	315 (10.4)	78 (10.7)	0.46
n-3 PUFAs intake (g/d)	0.6 (0.4)	0.7 (0.5)	<0.01
n-6 PUFAs intake (g/d)	4.7 (2.9)	5.4 (3.9)	<0.01

[1]Values represent mean ± SD, median (95% range) or number of subjects (valid%). n-3 and n-6 PUFAs intake was available in a subgroup of the study (n=2,313).
Abbreviations: PUFA; polyunsaturated fatty acid.

Table S3.2.2. Comparison of maternal PUFAs between those included and not included in the analyses (N = 6,924)[1]

Maternal PUFAs	Participation in follow-up N = 4,830	No participation in follow-up N = 2,094	*P*-value
Total PUFAs			
Absolute value (mg/L)	709.3 ± 97.9	702.0 ± 100.7	<0.01
Percentage by weight of total sum of fatty acids (%)	43.1 ± 2.0	43.4 ± 2.1	<0.01
Total n-3 PUFA			
Absolute value (mg/L)	105.0 ± 27.5	99.8 ± 26.6	<0.01
Percentage by weight of total sum of fatty acids (%)	6.4 ± 1.5	6.1 ±1.4	<0.01
ALA			
Absolute value (mg/L)	5.1 ± 1.9	4.8 ± 1.9	<0.01
Percentage by weight of total sum of fatty acids (%)	0.3 ± 0.1	0.3 ± 0.1	<0.01
EPA			
Absolute value (mg/L)	8.7 ± 5.4	7.8 ± 5.1	<0.01
Percentage by weight of total sum of fatty acids (%)	0.5 ± 0.3	0.5 ± 0.3	<0.01
DPA			
Absolute value (mg/L)	12.1 ± 4.3	11.6 ± 4.4	<0.01
Percentage by weight of total sum of fatty acids (%)	0.7 ± 0.2	0.7 ± 0.2	<0.01
DHA			
Absolute value (mg/L)	77.6 ± 20.4	74.1 ± 19.8	<0.01
Percentage by weight of total sum of fatty acids (%)	4.8 ± 1.1	4.6 ± 1.1	<0.01
Total n-6 PUFA			
Absolute value (mg/L)	604.2 ± 88.6	602.2 ± 90.8	0.38
Percentage by weight of total sum of fatty acids (%)	36.8 ± 2.5	37.2 ± 2.5	<0.01
LA			
Absolute value (mg/L)	361.9 ± 63.1	360.5 ± 62.2	0.41
Percentage by weight of total sum of fatty acids (%)	22.3 ± 2.8	22.6 ± 2.8	<0.01
GLA			
Absolute levels (mg/L)	1.5 ± 0.7	1.5 ± 0.7	0.35
Percentage by weight of total sum of fatty acids (%)	0.1 ± 0.0	0.1 ± 0.0	0.80
EDA			
Absolute value (mg/L)	8.5 ± 1.9	8.5 ± 1.9	0.05
Percentage by weight of total sum of fatty acids (%)	0.5 ± 0.1	0.5 ± 0.1	0.41
DGLA			
Absolute value (mg/L)	61.0 ± 16.5	59.2 ± 17.3	<0.01
Percentage by weight of total sum of fatty acids (%)	3.7 ± 0.7	3.7 ± 0.8	<0.01
AA			
Absolute levels (mg/L)	156.4 ± 32.6	157.3 ± 33.2	0.29
Percentage by weight of total sum of fatty acids (%)	9.6 ± 1.6	9.8 ± 1.6	<0.01
DTA			
Absolute value (mg/L)	6.9 ± 2.2	7.1 ± 2.2	<0.01
Percentage by weight of total sum of fatty acids (%)	0.4 ± 1.1	0.4 ± 0.1	<0.01
Total n-6/n-3 PUFAs ratio			
Absolute value (mg/L)	6.1 ± 1.7	6.4 ± 1.7	<0.01
Percentage by weight of total sum of fatty acids (%)	-	-	

[1]Values represent mean ± SD. Abbreviations: ALA: α-linolenic acid; AA: arachidonic acid; DGLA dihomo-gamma-linolenic acid; DHA: docosahexaenoic acid; DGLA dihomo-γ-linolenic acid; DPA: docosapentaenoic acid; DTA: docosatetraenoic acid; EDA: eicosadienoic acid; EPA: eicosapentaenoic acid; GLA: γ-linolenic acid; LA: linoleic acid; PUFA; polyunsaturated fatty acid.

Table S3.2.3. Correlation between maternal polyunsaturated fatty acid concentrations and child body fat outcomes (N = 4,830)[1]

Maternal PUFA	Total PUFA	Total n-3 PUFA	ALA	EPA	DPA	DHA	Total n-6 PUFA	LA	GLA	EDA	DGLA	AA	DTA	N6/n3 ratio	BMI	TFM	AvsG	PPAREA
Total PUFA	1																	
Total n-3 PUFA	0.46*	1																
ALA	0.37*	0.35*	1															
EPA	0.27*	0.82*	0.38*	1														
DPA	0.39*	0.69*	0.40*	0.60*	1													
DHA	0.44*	0.97*	0.22*	0.71*	0.54*	1												
Total n-6 PUFA	0.96*	0.21*	0.31*	0.06*	0.22*	0.19*	1											
LA	0.79*	0.06*	0.33*	-0.07*	0.08*	0.05*	0.86*	1										
GLA	0.36*	0.19*	0.23*	0.24*	0.30*	0.13*	0.34*	0.11*	1									
EDA	0.58*	0.12*	0.28*	-0.02	0.14*	0.11*	0.61*	0.60*	0.16*	1								
DGLA	0.59*	0.25*	0.32*	0.23*	0.36*	0.18*	0.58*	0.32*	0.53*	0.51*	1							
AA	0.63*	0.32*	0	0.19*	0.23*	0.34*	0.60*	0.20*	0.38*	0.11*	0.35*	1						
DTA	0.45*	0.05*	0.08*	-0.06*	0.33*	0	0.48*	0.16*	0.51*	0.28*	0.55*	0.58*	1					
N6/n3 ratio	0.06*	-0.84*	-0.17*	-0.75*	-0.54*	-0.81*	0.32*	0.39*	-0.01	0.21*	0.07*	0.01	0.21*	1				
BMI	0.01	-0.09*	-0.08*	-0.10*	-0.06*	-0.08*	0.04*	0.01	0.02	0.01	0.03	0.05*	0.07*	0.11*	1			
TFM	0.06*	-0.12*	-0.08*	-0.13*	-0.09*	-0.11*	0.10*	0.07*	0.05*	0.04*	0.06*	0.08*	0.09*	0.17*	0.57*	1		
AvsG	0.03	-0.09*	-0.06*	-0.10*	-0.08*	-0.08*	0.06*	0.05*	0.03	0.03*	0.05*	0.03	0.06*	0.12*	0.46*	0.55*	1	
PPAREA	0.07*	-0.08*	-0.08*	-0.09*	-0.05*	-0.07*	0.09*	0.06*	0.07*	0.03*	0.06*	0.09*	0.11*	0.13*	0.42*	0.55*	0.39*	1

[1]Values represent mean ± SD. Values are Pearson correlation coefficients. **P*-value <0.06

Abbreviations: AA: arachidonic acid; ALA: α-linolenic acid; AvsG: android/gynoid fat mass ratio; BMI: body mass index; DGLA: dihomo-γ-linolenic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; DTA: docosatetraenoic acid; EDA: eicosadienoic acid; EPA: eicosapentaenoic acid; GLA: γ-linolenic acid; LA: linoleic acid; PPAREA: abdominal preperitoneal fat mass; PUFA; polyunsaturated fatty acid; TFM: total fat mass.

Table S3.2.4. Associations of maternal n-3 PUFA concentrations with childhood body fat outcomes adjusted for pregnancy and childhood factors (N = 4,830)[1]

	Differences in childhood adiposity outcomes (95% confidence interval) in SDS			
Maternal n-3 PUFAs in SDS	**Body mass index (N = 4,830)**	**Total body fat mass percentage (N = 4,706)**	**Android/gynoid fat mass ratio (N = 4,706)**	**Preperitoneal fat mass area (N = 3,912)**
Total n-3 PUFAs				
Pregnancy factors[2]	-0.01 (-0.04, 0.01)	-0.07 (-0.10, -0.05)*	-0.07 (-0.10, -0.04)*	-0.02 (-0.06, 0.01)
Childhood factors[3]	-0.08 (-0.11, -0.05)*	-0.14 (-0.16, -0.11)*	-0.11 (-0.14, -0.08)*	-0.07 (-0.10, -0.04)*
ALA				
Pregnancy factors	-0.00 (-0.03, 0.02)	-0.02 (-0.05, 0.01)	-0.02 (-0.05, 0.01)	-0.03 (-0.06, 0.00)
Childhood factors	-0.07(-0.10, -0.04)*	-0.09 (-0.11, -0.06)*	-0.06 (-0.09, -0.04)*	-0.08 (-0.11, -0.05)*
EPA				
Pregnancy factors	-0.02 (-0.04, 0.01)	-0.06 (-0.08, -0.03)*	-0.05 (-0.08, -0.02)*	-0.02 (-0.05, 0.01)
Childhood factors	-0.07 (-0.10, -0.04)*	-0.11(-0.14, -0.09)*	-0.08 (-0.11, -0.05)*	-0.06 (-0.09, -0.03)*
DPA				
Pregnancy factors	0.00 (-0.03, 0.03)	-0.05 (-0.07, -0.02)*	-0.05 (-0.08, -0.02)*	0.01 (-0.02, 0.04)
Childhood factors	-0.05 (-0.08, -0.02)*	-0.10 (-0.12, -0.07)*	-0.08 (-0.11, -0.05)*	-0.03 (-0.06, -0.01)*
DHA				
Pregnancy factors	-0.01 (-0.04, 0.02)	-0.06 (-0.09, -0.04)*	-0.06 (-0.09, -0.03)*	-0.02 (-0.05, 0.01)
Childhood factors	-0.07 (-0.10, -0.04)*	-0.11 (-0.14, -0.09)*	-0.09 (-0.12, -0.07)*	-0.06 (-0.09, -0.03)*

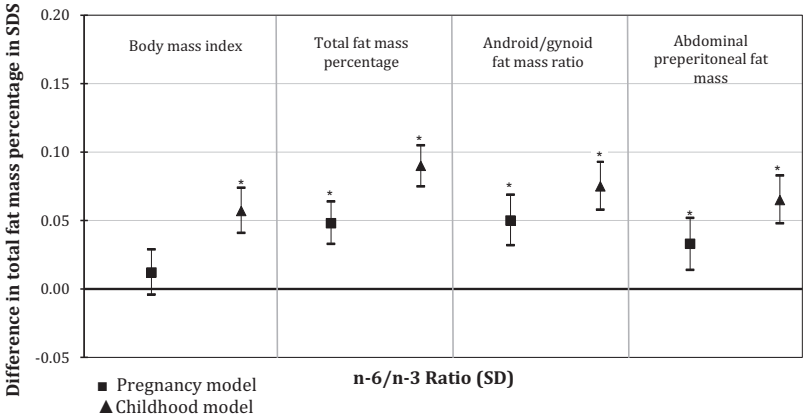
[1]Values are regression coefficients (95% CIs) that reflect the difference in SDS childhood body mass index, fat mass percentage, android/gynoid fat mass and abdominal preperitoneal fat mass per SD change in maternal n-3 PUFA concentrations, respectively.
[2]Pregnancy factors model are basic model additionally adjusted for maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, weight gain up to 30 weeks of gestation, blood pressure at enrolment, and smoking, folic acid supplement use and total calorie intake during pregnancy and pregnancy complications. [3]Childhood factors model are basic model additionally adjusted for birth characteristics, breastfeeding duration, timing of introduction of solid foods and TV watching time, respectively. * *P*-value<0.05.
Abbreviations: ALA: α-linolenic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; PUFA; polyunsaturated fatty acid; SD: standard deviation.

Table S3.2.5. Associations of maternal n-6 PUFA concentrations with childhood body fat outcomes adjusted for pregnancy and childhood factors (N = 4,830)[1]

	Differences in childhood adiposity outcomes (95% confidence interval) in SDS			
Maternal n-6 PUFAs in SDS	**Body mass index (N = 4,830)**	**Total body fat mass percentage (N = 4,706)**	**Android/gynoid fat mass ratio (N = 4,706)**	**Preperitoneal fat mass area (N = 3,912)**
Total n-6 PUFA				
Pregnancy factors[2]	0.02 (-0.01, 0.05)	0.08 (0.05, 0.10)*	0.06 (0.03, 0.10)*	0.07 (0.04, 0.11)*
Childhood factors[3]	0.10 (0.07, 0.13)*	0.15 (0.12, 0.18)*	0.11 (0.08, 0.14)*	0.13 (0.10, 0.16)*
LA				
Pregnancy factors	0.01 (-0.02, 0.04)	0.05 (0.02, 0.07)*	0.05 (0.02, 0.08)*	0.02 (-0.01, 0.05)
Childhood factors	0.03 (-0.00, 0.05)	0.07 (0.05, 0.10)*	0.06 (0.03, 0.09)*	0.05 (0.02, 0.08)*
GLA				
Pregnancy factors	0.00 (-0.03, 0.03)	0.02 (-0.01, 0.04)	0.00 (-0.03, 0.03)	0.04 (0.02, 0.07)*
Childhood factors	0.03 (-0.00, 0.05)	0.03 (0.01, 0.06)*	0.01 (-0.02, 0.04)	0.05 (0.02, 0.08)*
EDA				
Pregnancy factors	-0.01 (-0.03, 0.02)	-0.00 (-0.03, 0.02)	0.01 (-0.02, 0.04)	-0.01 (-0.04, 0.02)
Childhood factors	-0.00 (-0.03, 0.02)	0.02 (-0.01, 0.04)	0.02 (-0.01, 0.05)	0.01 (-0.02, 0.03)
DGLA				
Pregnancy factors	0.01 (-0.02, 0.04)	0.04 (0.02, 0.07)*	0.05 (0.02, 0.08)*	0.06(0.03, 0.10)*
Childhood factors	0.05 (0.02, 0.08)*	0.06 (0.04, 0.09)*	0.07 (0.04, 0.10)*	0.07 (0.04, 0.10)*
AA				
Pregnancy factors	0.00(-0.03, 0.03)	-0.00 (-0.03, 0.03)	-0.03 (-0.06, -0.00)*	0.03 (-0.00, 0.06)
Childhood factors	0.08 (0.05, 0.11)*	0.06 (0.03, 0.08)*	0.01 (-0.02, 0.04)	0.07 (0.04, 0.10)*
DTA				
Pregnancy factors	0.02 (-0.01, 0.05)	0.02 (-0.01, 0.05)	0.01 (-0.02, 0.04)	0.05 (0.02, 0.08)*
Childhood factors	0.08 (0.05, 0.10)*	0.06 (0.04, 0.09)*	0.05 (0.02, 0.08)*	0.08 (0.05, 0.11)*

[1]Values are regression coefficients (95% CIs) that reflect the difference in SDS childhood body mass index, fat mass percentage, android/gynoid fat mass and abdominal preperitoneal fat mass per SD change in maternal n-6 PUFA concentrations, respectively.
[2]Pregnancy factors model are basic model additionally adjusted for maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, weight gain up to 30 weeks of gestation, blood pressure at enrolment, and smoking, folic acid supplement use and total calorie intake during pregnancy and pregnancy complications. [3]Childhood factors model are basic model additionally adjusted for birth characteristics, breastfeeding duration, timing of introduction of solid foods and TV watching time, respectively.* *P*-value<0.05.
Abbreviations: AA: arachidonic acid; DGLA: dihomo-γ-linolenic acid; DTA: docosatetraenoic acid; GLA:γ-linolenic acid, EDA: eicosadienoic acid; LA: linoleic acid; PUFA; polyunsaturated fatty acid; SD: standard deviation.

Figure S3.2.2. Associations of maternal n-6/n-3 PUFAs ratio with childhood body fat outcomes adjusted for pregnancy and childhood factors (N = 4,830)



Values are regression coefficients (95% CIs) that reflect the difference in SDS of childhood body mass index, total body fat mass percentage, android/gynoid fat mass ratio and abdominal pre-peritoneal fat mass area per SD change in maternal n-6/n-3 PUFAs ratio, respectively. Basic models are adjusted for gestational age at blood sampling, child age, sex and height (fat mass outcomes only). Full models are adjusted for pregnancy factors and childhood factors, which included: maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, weight gain up to 30 weeks of gestation, blood pressure at enrolment, and smoking, folic acid supplement use and total calorie intake during pregnancy and pregnancy complications, gestational age and weight at birth, breastfeeding duration, timing of introduction of solid foods and TV watching time, respectively. * *P*-value<0.05. Abbreviations: PUFA; polyunsaturated fatty acid; SD: standard deviation.

Chapter 3.3

Maternal polyunsaturated fatty acids and childhood blood pressure

Aleksandra Jelena Vidakovic, Olta Gishti,
Jolien Steenweg - de Graaff, Michelle A. Williams,
Liesbeth Duijts, Janine F. Felix, Albert Hofman,
Henning Tiemeier, Vincent W.V. Jaddoe, Romy Gaillard

Adapted from J Nutr. 2015;145(10):2362-8.

Abstract

Background: Suboptimal maternal diet during pregnancy might lead to fetal cardiovascular adaptations with persistent consequences in the offspring.

Objectives: We assessed the associations of maternal polyunsaturated fatty acid (PUFA) concentrations during pregnancy with childhood blood pressure.

Methods: In a population-based prospective cohort study among 4,455 mothers and their children, we measured maternal second trimester n-3 and n-6 PUFA concentrations in plasma glycerophospholipids, and expressed n-3 and n-6 PUFAs as proportions of total PUFAs (weight percentage, wt%). Childhood blood pressure was measured at the median age of 6.0 years (95% range 5.7, 7.9). We used linear regression models to assess the associations of maternal PUFA wt% with childhood blood pressure at 6 years.

Results: Higher total maternal n-3 PUFA wt% and specifically DHA wt% were associated with lower childhood systolic blood pressure (differences: -0.28 mmHg (95% CI: -0.54, -0.03) and -0.29 mmHg (95% CI: -0.54, -0.03) per SD increase of total n-3 PUFA and DHA wt%, respectively), but not with childhood diastolic blood pressure. Total maternal n-6 PUFA wt% was positively associated with childhood systolic blood pressure (differences: 0.36 mmHg (95% CI: 0.09, 0.62) per SD increase of total n-6 PUFA wt%), but not with childhood diastolic blood pressure. A higher n-6/n-3 PUFA ratio was associated with higher childhood systolic blood pressure (*P* values<0.05). Pregnancy and childhood characteristics only partly explained the observed associations.

Conclusions: Higher maternal plasma n-3 PUFA and lower n-6 PUFA concentrations during pregnancy are associated with a lower systolic blood pressure in childhood. Further studies are needed to replicate these findings, to explore the underlying mechanisms and to examine the long term cardiovascular consequences.

Introduction

Suboptimal maternal and fetal nutrition might lead to fetal cardiovascular developmental maladaptations and a subsequent increased risk of cardiovascular disease in later life.[1] In humans, support for this hypothesis is largely based on historical cohort studies showing associations of maternal exposure to extreme famine during pregnancy with the development of hypertension in later life.[2] Not much is known about specific maternal nutritional factors during pregnancy, which may affect cardiovascular development in the offspring in less extreme environments. It has been hypothesized that maternal intake of long-chain polyunsaturated fatty acids (PUFAs) during pregnancy is important for fetal and infant development.[3,4] During pregnancy there is an increase in the demand for PUFAs due to the increased needs for maternal tissue and fetal development.[5] Lower maternal n-3 PUFA concentrations and higher n-6 PUFA concentrations have been associated with increased risks of gestational hypertensive disorders and low birth weight.[6-8] A previous study among 293 mother-child pairs showed that higher maternal plasma n-6 PUFA concentrations during pregnancy, but not n-3 PUFA concentrations, were associated with higher offspring fat mass.[9] In addition, a recent study in rats suggested that moderate n-3 PUFAs deficiency in the perinatal period was associated with hypertension in later life, despite reversal of the deficiency months before the assessment of blood pressure.[6] A study among Dutch children showed that children who received human milk with a relatively high concentration of n-3 PUFAs in the first year of life had a lower systolic blood pressure at the age of 12 years.[10] Thus far, no studies among humans have examined the associations of maternal PUFAs status during pregnancy with offspring blood pressure.

Therefore, we examined in a population-based prospective cohort study from early pregnancy onwards among 4,455 mothers and their children, the associations of maternal n-3 and n-6 PUFA plasma concentrations during pregnancy with childhood blood pressure.

Methods

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life to adulthood in Rotterdam, the Netherlands.[11] The study has been approved by the Medical Ethical Committee of the Erasmus Medical Center in Rotterdam (MEC 198.782/2001/31). All mothers gave written consent.

Pregnant women with an expected delivery date from April 2002 to January 2006 were enrolled in the study. In total, 8,879 mothers were enrolled during pregnancy, of whom 6,925 had PUFA concentrations available and gave birth to singleton live born children. Childhood blood pressure was available in 4,455 of these children (Flow chart is given **Supplemental Figure S3.3.1**).

Maternal polyunsaturated fatty acid status

Maternal non-fasting venous samples were drawn at a median gestational age of 20.6 weeks (95% range: 18.5-23.2). To analyze PUFA concentrations, EDTA plasma samples were picked and transported to the Division of Metabolic Diseases and Nutritional Medicine, Dr. von Hauner Children's Hospital, University of Munich Medical Center. After being thawed, the analysis of plasma glycerophospholipid PUFAs composition was performed by a sensitive and precise high-throughput method, suitable in large epidemiological studies, as previously described.[12] Based on findings from previous studies, we selected maternal PUFAs for our analyses, which have been associated with the risk of cardiovascular disease in adults, and maternal and fetal pregnancy outcomes.[8,9,13-16] Selected maternal PUFAs were total n-3 PUFA, which included α-linolenic (ALA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and total n-6 PUFA, which included linoleic acid (LA, 18:2n-6), γ-linolenic acid (GLA, 18:3n-6), eicosadienoic acid (EDA, 20:2n-6), dihomo-γ-linolenic acid (DGLA, 20:3n-6), arachidonic acid (AA, 20:4n-6), docosatetraenoic acid (DTA, 22:4n-6). The ratio of total n-6/n-3 PUFA was calculated. PUFA concentrations were expressed as proportion of total PUFAs present in the chromatogram (weight percentage, wt%).[17] We observed similar results when we used fatty acid concentrations in mg/L instead of percentages (results not shown).

Childhood blood pressure

We measured blood pressure at the median age of 6.0 years (95% range: 5.7, 7.9) four times with one-minute intervals at the right brachial artery with the child in supine position, using the validated automatic sphygmanometer Datascope Accutor Plus TM (Paramus, NJ, USA).[18] A cuff was selected with a cuff width approximately 40% of the arm circumference and long enough to cover 90% of the arm circumference. We used mean systolic and diastolic blood pressure values calculated from the last three blood pressure measurements.

Covariates

Information on maternal age, educational level, ethnicity and maternal folic acid supplement use was obtained at enrolment.[11] We measured maternal height, and blood pressure at enrolment, and obtained information about maternal prepregnancy weight by questionnaire. We calculated body mass index. Maternal weight gain during pregnancy was calculated as the difference between maternal weight at third trimester and maternal prepregnancy weight. Information on maternal smoking and alcohol consumption was assessed by questionnaires during pregnancy. Homocysteine and folate concentrations were analysed using an immunoelectrochemoluminescence assay on the Architect System (Abbott Diagnostics B.V., Hoofddorp, the Netherlands). We used food frequency questionnaires to assess maternal nutritional information during early pregnancy at the median gestational age 13.8 weeks (95% range: 9.9, 22.6). We also assessed infant PUFA intakes at 13 months by a FFQ questionnaire in a subgroup of the study cohort (n=2,153).[19] Information about pregnancy complications, childhood sex, gestational age, and weight at birth was obtained from medical records.[20,21] Information about breastfeeding and timing of introduction of solid foods was obtained by questionnaires in infancy.[19] At the age of 6 years, childhood height and weight were measured, and body mass index was calculated.

Statistical analysis

We examined the associations of maternal PUFA wt%, expressed as standard deviation difference, with childhood systolic and diastolic blood pressure using linear regression models. We used four regression models; (1) a basic model including gestational age at maternal blood sampling, child's age and sex; (2) a pregnancy factor adjusted model, which was additionally adjusted for maternal age, educational level, ethnicity, parity, prepregnancy body mass index, gestational weight gain, blood pressure at enrolment, smoking, alcohol consumption, folic acid and homocysteine concentrations in plasma, total caloric intake during pregnancy, and pregnancy complications; (3) a childhood factor adjusted model, which was the basic model additionally adjusted for gestational age and weight at birth, breastfeeding duration, timing of introduction of solid foods and childhood current body mass index; (4) and a fully adjusted model which includes all the above factors. Included covariates were selected based on their associations with the outcomes of interest based on previous studies [9] or a change in effect estimate of >10%. We performed a sensitivity analysis, among a subgroup of children with information about PUFA intakes at 13 months available, by additional adjusting the performed analyses for PUFA intakes in infancy. We tested for interaction terms between child's sex and maternal PUFA wt% in re-

lation to blood pressure in childhood. Since no significant interaction terms were present, no further stratified analyses were performed. In order to reduce potential bias associated with missing data and to maintain statistical power, we performed multiple imputations of missing covariates by generating 5 independent datasets using the Markov Chain Monte Carlo method after which the pooled effect estimates were calculated. All analyses were performed using Statistical Package for the Social Sciences version 21.0 for Windows (SPSS Inc, Chicago, IL, USA). Values represent means ±standard deviations (SD), medians (95% range), numbers (valid percentage) or estimates (95% Confidence Interval, CI) obtained from linear regression models. We used the 5% level of significance.

Results

Maternal and childhood characteristics are given in **Table 3.3.1.** Second trimester maternal total n-3 and n-6 PUFA concentrations were 104 ± 27.5 mg/L and 597 ± 87.5 mg/L, respectively **(Table 3.3.2)**. Results from the non-response analysis are given in **Supplemental Table S3.3.1** and showed that mothers who were not included in the analyses smoked more often during pregnancy and developed more often preeclampsia as compared to those who were included (*P* values<0.05). Also, birth weight, gestational age at birth and breastfeeding duration were lower among children who were not included in the analyses compared to those who were included (*P* values<0.05).

Table 3.3.1. Characteristics of mothers and their children (N =4,455)[1]

Maternal characteristics	**Value**
Age, (yr)	30.9 (20.0, 39.2)
Gestational age at enrolment, (wk)	13.8 (9.9-22.6)
Prepregnancy body mass index, (kg/m^2)	23.6 ± 4.2
Weight gain during pregnancy, (kg)	10.4 ± 4.9
Systolic blood pressure at intake, (mmHg)	116 ± 12.2
Diastolic blood pressure at intake, (mmHg)	68.1 ± 9.5
Education, No. higher education (%)	1938 (46.2)
Race / ethnicity, No. European (%)	2683 (61.3)
Parity, No. nulliparous (%)	1897 (42.9)
Total energy intake, (kcal/d)	2051 ± 559
Smoking during pregnancy, No. Yes (%)	1037 (26.1)
Alcohol consumption during pregnancy, No. Yes (%)	2150 (54.7)
Plasma folate, (nmol/L)	18.5 ± 9.0
Plasma homocysteine, (μmol/L)	7.1 ± 1.9
Preeclampsia, No. (%)	80 (1.9)
Gestational hypertension, No. (%)	170 (4.0)
Gestational diabetes, No. (%)	40 (0.9)
Childhood characteristics	
Total PUFAs intake, (g/d)	7.9 (0.64, 38.4)
N-6 PUFA intake, (g/d)	4.1 (0.03-28.9)
N-3 PUFA intake, (g/d)	0.5 (0.00, 3.67)
Males, No. (%)	2225 (49.9)
Gestational age at birth, (wk)	40.1 (35.7, 42.4)
Birth weight, (g)	3436 ± 551
Ever breastfeeding, Yes (%)	3275 (92.8)
Introduction of solid foods (%)	
< 3 months	201 (8.3)
3-4.9 months	1484 (60.6)
>5 months	762 (31.1)
Age at follow up, (yr)	6.0 (5.7, 7.9)
Body mass index, (kg/m^2)	15.8 (13.6, 21.2)
Systolic blood pressure, (mmHg)	102.6 ± 8.2
Diastolic blood pressure, (mmHg)	60.6 ± 6.9

[1]Values represent means ± SDs, median (95% range) or number of subjects (valid%).

Table 3.3.2. Plasma PUFA concentrations during the second trimester of mothers (N =4,455)[1]

	Absolute value (mg/L)	Relative value (wt%)
Total PUFAs	700 ± 96.8	-
Total n-3 PUFAs	104 ± 27.5	6.4 ± 1.5
18:3n-3	5.1 ± 1.9	0.3 ± 0.1
20:5n-3	8.7 ± 5.4	0.5 ± 0.3
22:5n-3	12.1 ± 4.3	0.7 ± 0.2
22:6n-3	77.6 ± 20.5	4.8 ± 1.1
Total n-6 PUFAs	597 ± 87.5	36.8 ± 2.5
18:2n-6	362 ± 63.5	22.3 ± 2.8
18:3n-6	1.5 ± 0.7	0.1 ± 0.0
20:2n-6	8.5 ± 1.9	0.5 ± 0.1
20:3n-6	61.1 ± 16.5	3.7 ± 0.7
20:4n-6	157 ± 32.6	9.6 ± 1.6
22:4n-6	7.0 ± 2.2	0.4 ± 1.1
Total n-6/n-3 PUFA ratio	6.1 ± 1.7	-

[1]Values represent means ± SDs, PUFAs: polyunsaturated fatty acids

Maternal n-3 PUFA wt% and childhood blood pressure

Table 3.3.3 shows that in the models adjusted for gestational age at blood sampling, child age and sex, higher total n-3 PUFA wt% and specifically higher ALA, EPA, DPA and DHA wt% were associated with lower childhood systolic blood pressure (*P* values <0.05). Additional adjustment for pregnancy and childhood factors fully explained the associations of ALA, EPA, DPA wt% with childhood systolic blood pressure. In the fully adjusted models, higher maternal total n-3 PUFA and DHA wt% were associated with lower childhood systolic blood pressure (differences: -0.28 mmHg (95% CI:-0.54, -0.03) and -0.29 mmHg (95% CI: -0.54, -0.03) per SD increase of total maternal n-3 PUFA and DHA wt%, respectively). In the basic and childhood models, higher maternal total n-3 PUFA, DPA and DHA wt% were associated with lower childhood diastolic blood pressure (*P* values<0.05), but no associations were present in the pregnancy and fully adjusted models.

Table 3.3.3. Associations of maternal n-3 PUFA wt% with blood pressure in children at 6 years (N = 4,455)[1]

	Differences in childhood systolic and diastolic blood pressure (95% confidence interval) per SD difference in n-3 PUFAs				
	Total n-3 PUFAs (SD)	**18:3n-3 (SD)**	**20:5n-3 (SD)**	**22:5n-3 (SD)**	**22:6n-3 (SD)**
Systolic blood pressure (mmHg)					
Basic model[2]	-0.60 (-0.84, -0.36)*	-0.32 (-0.56, -0.08)*	-0.30 (-0.54, -0.06)*	-0.42 (-0.66, -0.17)*	-0.58 (-0.82, -0.34)*
Pregnancy model[3]	-0.33 (-0.59, -0.07)*	-0.12 (-0.37, 0.13)	-0.07 (-0.32, 0.18)	-0.24 (-0.48, 0.01)	-0.33 (-0.59, -0.07)*
Childhood model[4]	-0.35 (-0.58, -0.11)*	-0.15 (-0.38, 0.09)	-0.08 (-0.31, 0.15)	-0.26 (-0.50, -0.03)*	-0.37 (-0.59, -0.12)*
Full model[5]	-0.28 (-0.54, -0.03)*	-0.12 (-0.36, 0.12)	-0.03 (-0.28, 0.22)	-0.22 (-0.46, 0.02)	-0.29 (-0.54, -0.03)*
Diastolic blood pressure (mmHg)					
Basic model	-0.38 (-0.58, -0.17)*	-0.19 (-0.40, 0.00)	-0.12 (-0.32, 0.08)	-0.35 (-0.55, -0.15)*	-0.36 (-0.57, -0.16)*
Pregnancy model	-0.11 (-0.33, 0.11)	-0.04 (-0.25, 0.17)	0.12 (-0.09, 0.34)	-0.19 (-0.40, 0.02)	-0.13 (-0.35, 0.09)
Childhood model	-0.26 (-0.47, -0.06)*	-0.13 (-0.33, 0.07)	-0.02 (-0.22, 0.18)	-0.28 (-0.48, -0.07)*	-0.27 (-0.47, -0.06)*
Full model	-0.10 (-0.32, 0.12)	-0.04 (-0.25, 0.17)	0.13 (-0.08, 0.34)	-0.18 (-0.39, 0.03)	-0.12 (-0.34, 0.10)

[1]Values are regression coefficients (95% CIs) obtained from linear regression models and reflect the difference in childhood blood pressure levels in mmHg per SD difference in each maternal n-3 PUFAs respectively. [2]Basic models are adjusted for gestational age at blood sampling, child age and sex. [3]Pregnancy models are basic models additionally adjusted for maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, gestational weight gain, blood pressure at enrolment, smoking, alcohol consumption, folic acid and homocysteine concentrations in plasma, total caloric intake during pregnancy, and pregnancy complications. [4]Childhood models are basic models additionally adjusted for birth characteristics, breastfeeding duration, timing of introduction of solid foods and body mass index at the age of 6 years, respectively. [5]Full models are adjusted for all covariates. Abbreviations: CIs: Confidence Intervals; PUFAs: polyunsaturated fatty acids; SD: standard deviation. * P-value<0.05.

Maternal n-6 PUFA wt% and childhood blood pressure

Table 3.3.4 shows that in the basic model, higher total n-6 PUFA, AA and DTA wt% were associated with a higher childhood systolic blood pressure (*P* values<0.05). Additional adjustment for pregnancy or childhood factors partly explained these associations. In the fully adjusted model, only higher total maternal n-6 PUFA wt% was associated with a higher childhood systolic blood pressure (difference: 0.36 mmHg (95% CI: 0.09, 0.62) per SD increase of total n-6 PUFA wt%). In the basic model higher maternal total n-6 PUFA, AA and DTA wt% were associated with a higher childhood diastolic blood pressure (*P* values<0.05), whereas in the childhood model only higher total maternal n-6 PUFA and DTA wt% were associated with childhood diastolic blood pressure (*P* values<0.05). No associations were present in the pregnancy and fully adjusted models.

Table 3.3.4. Associations of maternal n-6 PUFA wt% with blood pressure in children at 6 years (N = 4,455)[1]

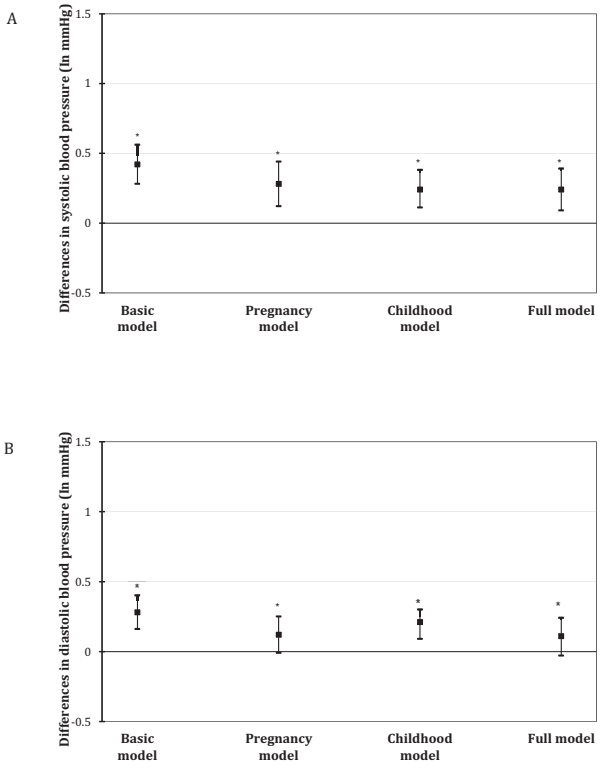
	Differences in childhood systolic and diastolic blood pressure (95% confidence interval) per SD difference in n-6 PUFAs						
	Total n-6 PUFAs (SD)	**18:2n-6 (SD)**	**18:3n-6 (SD)**	**20:2n-6 (SD)**	**20:3n-6 (SD)**	**20:4n-6 (SD)**	**22:4n-6 (SD)**
Systolic blood pressure (mmHg)							
Basic model[2]	0.62 (0.38, 0.86)*	0.22 (-0.02, 0.46)	0.22 (-0.02, 0.46)	0.01 (-0.22, 0.25)	0.23 (-0.01, 0.47)	0.42 (0.18, 0.66)*	0.45 (0.21, 0.69)*
Pregnancy model[3]	0.41 (0.14, 0.68)*	0.23 (-0.03, 0.48)	0.09 (-0.15, 0.33)	0.03 (-0.21, 0.28)	0.04 (-0.22, 0.29)	0.11 (-0.14, 0.36)	0.19 (-0.06, 0.44)
Childhood model[4]	0.33 (0.09, 0.56)*	0.12 (-0.12, 0.35)	0.17 (-0.06, 0.40)	-0.03 (-0.23,0.23)	0.12 (-0.12, 0.35)	0.22 (-0.02, 0.45)	0.24 (0.00, 0.47)*
Full model[5]	0.36 (0.09, 0.62)*	0.20 (-0.05, 0.45)	0.10 (-0.13, 0.34)	0.05 (-0.19, 0.29)	0.01 (-0.24, 0.26)	0.11 (-0.14, 0.36)	0.15 (-0.10, 0.40)
Diastolic blood pressure (mmHg)							
Basic model	0.37 (0.17, 0.58)*	0.14 (-0.06, 0.34)	0.17 (-0.02, 0.38)	0.06 (-0.14, 0.27)	0.17 (-0.03, 0.37)	0.21 (0.01, 0.42)*	0.40 (0.20, 0.61)*
Pregnancy model	0.10 (-0.13, 0.33)	0.04 (-0.18, 0.25)	0.08 (-0.13, 0.28)	0.01 (-0.19, 0.22)	0.10 (-0.11, 0.31)	0.01 (-0.21, 0.22)	0.17 (-0.04, 0.38)
Childhood model	0.24 (0.04, 0.45)*	0.09 (-0.11, 0.29)	0.16 (-0.04, 0.36)	0.06 (-0.14, 0.26)	0.16 (-0.04, 0.37)	0.11 (-0.09, 0.31)	0.31 (0.11, 0.51)*
Full model	0.09 (-0.14, 0.32)	0.04 (-0.17, 0.26)	0.09 (-0.17, 0.29)	0.03 (-0.18, 0.23)	0.10 (-0.11, 0.31)	-0.02 (-0.23,0.19)	0.16 (-0.05, 0.37)

[1]Values are regression coefficients (95% CIs) obtained from linear regression models and reflect the difference in childhood blood pressure levels in mmHg per SD difference in each maternal n-6 PUFAs respectively. [2]Basic models are adjusted for gestational age at blood sampling, child age and sex. [3]Pregnancy models are basic models additionally adjusted for maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, gestational weight gain, blood pressure at enrolment, smoking, alcohol consumption, folic acid and homocysteine concentrations in plasma, total caloric intake during pregnancy, and pregnancy complications. [4]Childhood models are basic models additionally adjusted for birth characteristics, breastfeeding duration, timing of introduction of solid foods and body mass index at the age of 6 years, respectively. [5]Full models are adjusted for all covariates. Abbreviations: CIs: Confidence Intervals; PUFAs: polyunsaturated fatty acids; SD: standard deviation. * P-value<0.05.

Maternal n-6/n-3 PUFA ratio and childhood blood pressure

Figure 3.3.1 shows that higher maternal n-6/n-3 PUFA ratio was associated with a higher childhood systolic and diastolic blood pressure in the basic models (p-values<0.05). Adjustment for pregnancy and childhood factors separately slightly attenuated these associations. In the fully adjusted model, higher maternal n-6/n-3 PUFA ratios were associated with an increased childhood systolic blood pressure (differences: 0.24 mmHg (95% CI: 0.09, 0.39) per SD increase of n-6/n-3 PUFA ratio), but not with diastolic blood pressure. Additional adjustment for infants' PUFA intakes at 13 months did not affect the observed associations (data not shown).

Figure 3.3.1. Associations between the maternal plasma phospholipid n-6/n-3 PUFA ratio with systolic (A) and diastolic (B) blood pressure in children at 6 years (N=4,455) [1]



[1]Values are regression coefficients (95% CIs) obtained from linear regression models and reflect the differences in childhood blood pressure level per SD difference in maternal n-6/n-3 PUFA ratio. Basic models are adjusted for gestational age at blood sampling, child age and sex. Pregnancy models are basic models additionally adjusted for maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, gestational weight gain, blood pressure at enrolment, smoking, alcohol consumption, folic acid and homocysteine concentrations in plasma, total caloric intake during pregnancy, and pregnancy complications. Childhood models are basic models additionally adjusted birth characteristics, breastfeeding duration, timing of introduction of solid foods and body mass index at the age of 6 years, respectively. Full models are adjusted for all covariates. * P-value<0.05.

Discussion

In this population based prospective cohort study, we observed that higher maternal plasma n-3 PUFA (total and DHA) and lower n-6 PUFA concentrations during pregnancy were associated with a lower systolic blood pressure in school age children. These associations were only partly explained by maternal and childhood characteristics.

Methodological considerations

We used a population-based prospective cohort study design with a large number of subjects. Of all children in whose maternal PUFA concentrations were available, 64% participated in the cardiovascular follow-up studies. The non-response could lead to biased effect estimates if the associations of maternal PUFA concentrations with childhood blood pressure would be different between children included and not included in the analyses. Non-response analysis showed various differences between subjects included and not included in the analyses. It is hard to speculate whether these differences would affect the observed associations materially. Although we measured a large number of maternal PUFA concentrations in blood samples, they were measured only once during pregnancy. No information was available about PUFA concentrations in early or late pregnancy. Further studies are needed to examine critical periods of maternal PUFA status for offspring outcomes. Also, concentrations of maternal PUFA may not fully reflect the concentrations of PUFA that the fetus was exposed to, as this also depends on placental transfer.[42] Finally, although we performed an extensive adjustment for a large number of potential confounders, residual confounding might still be an issue, as in any observational study. Most importantly, we did not have detailed information about maternal dietary intake during pregnancy or on childhood diet or PUFA concentrations available in the full cohort. We assessed maternal dietary intake during first trimester by a FFQ, but we did not have information about maternal diet during second trimester available. We were able to adjust for childhood intakes of PUFA in a subgroup of the study population and observed that the associations were not materially affected. Further studies are needed to explore the role of the potential confounding by maternal and childhood dietary factors in these observed associations.

Interpretation of main findings

Suboptimal nutrition in early life is associated with an increased risk of cardiovascular diseases in later life.[22,23] Multiple studies have shown that maternal intake of long-chain PUFAs during pregnancy is important for fetal development.[4,5] Not much is known about the associations of maternal PUFA concentrations during pregnancy with offspring cardiovascular and metabolic outcomes in later life.

To our knowledge, only a few studies examined the associations of maternal PUFA concentrations during pregnancy with cardiovascular outcomes in the offspring. A study among 1,250 mother-child pairs showed that higher maternal n-3 PUFA intake was associated with lower adiposity levels in children at age 3 years, whereas a higher n-6/n-3 PUFA ratio in the maternal diet was associated with higher child adiposity levels.[13] Moreover, a study among 293 mother-child pairs showed that higher

maternal plasma concentrations of total n-6 PUFA, LA and AA during pregnancy were associated with an increased risk of obesity and higher fat mass levels in children aged 4 and 6 years.[9] However, a small randomized controlled trial among 208 healthy pregnant women did not find a beneficial effect of the reduction of the n-6/n-3 PUFA ratio in the maternal diet on adipose tissue level in infants.[24] In our current study, we observed that higher maternal plasma n-3 PUFA and lower n-6 PUFA concentrations during pregnancy were associated with lower systolic blood pressure in childhood. Of all specific PUFAs, the strongest associations were present for DHA and DTA. These associations were only partly explained by maternal and childhood characteristics.

We are not aware of other published studies focused on the associations of maternal PUFA concentrations during pregnancy with childhood blood pressure in humans. A study in rats showed that n-3 PUFAs deficiency in the perinatal period leads to a higher blood pressure later in life.[25] A review of animal experiments, human observational studies and randomized clinical trials has shown that the intake of n-3 PUFAs was associated with a lower systolic and diastolic blood pressure in adults and with a reduced risk of coronary heart disease.[26] Also, a meta-analysis of clinical trials suggested that fish oil reduced blood pressure levels in humans.[27] In children, the effects of n-3 and n-6 PUFAs intake on blood pressure have been examined in early postnatal life. A European study among 147 children from the United Kingdom, Belgium and Italy showed that children who as infants had been fed with a formula supplemented with AA and DHA had lower mean systolic blood pressure and diastolic blood pressure at the age of 6 years than those who were not supplemented.[28] A randomized trial among 83 Danish children at the age of 9 months showed that systolic blood pressure was lower among children who got fish oil supplementation for three months.[29] A study among 973 Dutch children showed that children who received human milk with a relatively high content of DHA and EPA had lower systolic blood pressure at the age of 12 years. This association was not influenced from total n-3 PUFAs measured in erythrocyte membrane at the age of 12 years.[10] In line with these studies, our current study suggests that maternal n-3 and n-6 PUFA concentrations may already influence cardiovascular development from early fetal life onwards.

Several potential mechanisms may explain the observed associations of maternal n-3 and n-6 PUFA concentrations during pregnancy with childhood blood pressure.[13,23,30-33] N-3 PUFAs in fish oil are shown to have hypotensive properties through stimulation of prostaglandins that control sodium and water excretion, inhibition of the vasoconstrictor thromboxane and decrease of the response to vasopressor hormones.[34-36] In contrary, a higher n-6 PUFAs intake can inhibit the conversion of ALA to EPA, which can downregulate the production of prostaglandine PGE2, which induces

vascular relaxation.[36,37] N-6 PUFAs are precursors of the potent 2-series prostaglandins, and the vasoconstrictor thromboxane A2, which can stimulate vasoconstriction.[38] N-3 PUFAs are also shown to affect the expression of inflammatory genes and may reduce the production of pro-inflammatory leukotrienes and cytokines.[39] It has been suggested that specifically the balance of n-3 and n-6 PUFAs is important for optimal cardiovascular development.[40,41] Our findings support the hypothesis that lowering of the n-6/n-3 PUFAs ratio during pregnancy might have beneficial health effects in the offspring.[40,41] Yet, further studies are needed to examine whether the observed associations are indeed causal or reflect confounding by family based socio-demographic and lifestyle related characteristics, to further explore the underlying mechanisms and to examine whether they persist into adulthood.

Conclusions

Suboptimal maternal diet during pregnancy might lead to fetal cardiovascular adaptations with persistent cardiovascular consequences in later life. Maternal intake of PUFAs during pregnancy is important for fetal and infant development. We observed that higher maternal plasma n-3 PUFA and lower n-6 PUFA concentrations during pregnancy are associated with a lower systolic blood pressure in childhood. These results should be considered as hypothesis generating. Further observational and experimental studies are needed to explore the underlying mechanisms and to examine the long term cardiovascular consequences.

References

1. Barker DJ. Fetal origins of coronary heart disease. BMJ 1995;311(6998):171-174.
2. Roseboom TJ, van der Meulen JH, Ravelli AC, Osmond C, Barker DJ, Bleker OP. Effects of prenatal exposure to the Dutch famine on adult disease in later life: an overview. Mol Cell Endocrinol 2001;185(1-2):93-98.
3. Szajewska H, Horvath A, Koletzko B. Effect of n-3 long-chain polyunsaturated fatty acid supplementation of women with low-risk pregnancies on pregnancy outcomes and growth measures at birth: a meta-analysis of randomized controlled trials. Am J Clin Nutr 2006;83(6):1337-1344.
4. Cetin I, Koletzko B. Long-chain omega-3 fatty acid supply in pregnancy and lactation. Curr Opin Clin Nutr Metab Care 2008;11(3):297-302.
5. Koletzko B, Agostoni C, Carlson SE, Clandinin T, Hornstra G, Neuringer M, Uauy R, Yamashiro Y, Willatts P. Long chain polyunsaturated fatty acids (LC-PUFA) and perinatal development. Acta Paediatr 2001;90(4):460-464.
6. Armitage JA, Pearce AD, Sinclair AJ, Vingrys AJ, Weisinger RS, Weisinger HS. Increased blood pressure later in life may be associated with perinatal n-3 fatty acid deficiency. Lipids 2003;38(4):459-464.

7. McFadyen M, Farquharson J, Cockburn F. Maternal and umbilical cord erythrocyte omega-3 and omega-6 fatty acids and haemorheology in singleton and twin pregnancies. Arch Dis Child Fetal Neonatal Ed 2003;88(2):F134-138.
8. Mahomed K, Williams MA, King IB, Mudzamiri S, Woelk GB. Erythrocyte omega-3, omega-6 and trans fatty acids in relation to risk of preeclampsia among women delivering at Harare Maternity Hospital, Zimbabwe. Physiol Res 2007;56(1):37-50.
9. Moon RJ, Harvey NC, Robinson SM, Ntani G, Davies JH, Inskip HM, Godfrey KM, Dennison EM, Calder PC, Cooper C, Group SWSS. Maternal plasma polyunsaturated fatty acid status in late pregnancy is associated with offspring body composition in childhood. J Clin Endocrinol Metab 2013;98(1):299-307.
10. van Rossem L, Wijga AH, de Jongste JC, Koppelman GH, Oldenwening M, Postma DS, Abrahamse-Berkeveld M, van de Heijning B, Brunekreef B, Smit HA. Blood pressure in 12-year-old children is associated with fatty acid composition of human milk: the prevention and incidence of asthma and mite allergy birth cohort. Hypertension 2012;60(4):1055-1060.
11. Jaddoe VW, van Duijn CM, Franco OH, van der Heijden AJ, van Iizendoorn MH, de Jongste JC, van der Lugt A, Mackenbach JP, Moll HA, Raat H, Rivadeneira F, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC, Hofman A. The Generation R Study: design and cohort update 2012. Eur J Epidemiol 2012;27(9):739-756.
12. Glaser C, Demmelmair H, Koletzko B. High-throughput analysis of fatty acid composition of plasma glycerophospholipids. J Lipid Res 2010;51(1):216-221.
13. Donahue SM, Rifas-Shiman SL, Gold DR, Jouni ZE, Gillman MW, Oken E. Prenatal fatty acid status and child adiposity at age 3 y: results from a US pregnancy cohort. Am J Clin Nutr 2011;93(4):780-788.
14. Koletzko B, Lien E, Agostoni C, Bohles H, Campoy C, Cetin I, Decsi T, Dudenhausen JW, Dupont C, Forsyth S, Hoesli I, Holzgreve W, Lapillonne A, Putet G, Secher NJ, Symonds M, Szajewska H, Willatts P, Uauy R, World Association of Perinatal Medicine Dietary Guidelines Working G. The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. J Perinat Med 2008;36(1):5-14.
15. Kinsella JE, Lokesh B, Stone RA. Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. Am J Clin Nutr 1990;52(1):1-28.
16. de Groot RH, Hornstra G, van Houwelingen AC, Roumen F. Effect of alpha-linolenic acid supplementation during pregnancy on maternal and neonatal polyunsaturated fatty acid status and pregnancy outcome. Am J Clin Nutr 2004;79(2):251-260.
17. Jaddoe VW, Bakker R, van Duijn CM, van der Heijden AJ, Lindemans J, Mackenbach JP, Moll HA, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC, Hofman A. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. Eur J Epidemiol 2007;22(12):917-923.
18. Wong SN, Tz Sung RY, Leung LC. Validation of three oscillometric blood pressure devices against auscultatory mercury sphygmomanometer in children. Blood Press Monit 2006;11(5):281-291.
19. Heppe DH, Medina-Gomez C, Hofman A, Franco OH, Rivadeneira F, Jaddoe VW. Maternal first-trimester diet and childhood bone mass: the Generation R Study. Am J Clin Nutr 2013;98(1):224-232.
20. Verburg BO, Steegers EA, De Ridder M, Snijders RJ, Smith E, Hofman A, Moll HA, Jaddoe VW, Witteman JC. New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. Ultrasound Obstet Gynecol 2008;31(4):388-396.

21. Niklasson A, Ericson A, Fryer JG, Karlberg J, Lawrence C, Karlberg P. An update of the Swedish reference standards for weight, length and head circumference at birth for given gestational age (1977-1981). Acta Paediatr Scand 1991;80(8-9):756-762.
22. Roseboom TJ, van der Meulen JH, Ravelli AC, Osmond C, Barker DJ, Bleker OP. Effects of prenatal exposure to the Dutch famine on adult disease in later life: an overview. Twin Res 2001;4(5):293-298.
23. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med 2008;359(1):61-73.
24. Hauner H, Much D, Vollhardt C, Brunner S, Schmid D, Sedlmeier EM, Heimberg E, Schuster T, Zimmermann A, Schneider KT, Bader BL, Amann-Gassner U. Effect of reducing the n-6:n-3 long-chain PUFA ratio during pregnancy and lactation on infant adipose tissue growth within the first year of life: an open-label randomized controlled trial. Am J Clin Nutr 2012;95(2):383-394.
25. Weisinger HS, Armitage JA, Sinclair AJ, Vingrys AJ, Burns PL, Weisinger RS. Perinatal omega-3 fatty acid deficiency affects blood pressure later in life. Nat Med 2001;7(3):258-259.
26. Mozaffarian D, Wu JH. Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. J Am Coll Cardiol 2011;58(20):2047-2067.
27. Geleijnse JM, Giltay EJ, Grobbee DE, Donders AR, Kok FJ. Blood pressure response to fish oil supplementation: metaregression analysis of randomized trials. J Hypertens 2002;20(8):1493-1499.
28. Forsyth JS, Willatts P, Agostoni C, Bissenden J, Casaer P, Boehm G. Long chain polyunsaturated fatty acid supplementation in infant formula and blood pressure in later childhood: follow up of a randomised controlled trial. BMJ 2003;326(7396):953.
29. Damsgaard CT, Schack-Nielsen L, Michaelsen KF, Fruekilde MB, Hels O, Lauritzen L. Fish oil affects blood pressure and the plasma lipid profile in healthy Danish infants. J Nutr 2006;136(1):94-99.
30. Hauner H, Vollhardt C, Schneider KT, Zimmermann A, Schuster T, Amann-Gassner U. The impact of nutritional fatty acids during pregnancy and lactation on early human adipose tissue development. Rationale and design of the INFAT study. Ann Nutr Metab 2009;54(2):97-103.
31. Ciccone MM, Scicchitano P, Gesualdo M, Zito A, Carbonara S, Ricci G, Cortese F, Giordano P. The role of omega-3 polyunsaturated fatty acids supplementation in childhood: a review. Recent Pat Cardiovasc Drug Discov 2013;8(1):42-55.
32. Ailhaud G, Guesnet P. Fatty acid composition of fats is an early determinant of childhood obesity: a short review and an opinion. Obes Rev 2004;5(1):21-26.
33. Farvid MS, Ding M, Pan A, Sun Q, Chiuve SE, Steffen LM, Willett WC, Hu FB. Dietary Linoleic Acid and Risk of Coronary Heart Disease: A Systematic Review and Meta-Analysis of Prospective Cohort Studies. Circulation 2014.
34. Morris MC, Sacks F, Rosner B. Does fish oil lower blood pressure? A meta-analysis of controlled trials. Circulation 1993;88(2):523-533.
35. Cicero AF, Ertek S, Borghi C. Omega-3 polyunsaturated fatty acids: their potential role in blood pressure prevention and management. Curr Vasc Pharmacol 2009;7(3):330-337.
36. Harris WS, Shearer GC. Omega-6 Fatty Acids and Cardiovascular Disease: Friend or Foe? Circulation 2014.
37. Liou YA, King DJ, Zibrik D, Innis SM. Decreasing linoleic acid with constant alpha-linolenic acid in dietary fats increases (n-3) eicosapentaenoic acid in plasma phospholipids in healthy men. J Nutr 2007;137(4):945-952.

38. Patterson E, Wall R, Fitzgerald GF, Ross RP, Stanton C. Health implications of high dietary omega-6 polyunsaturated Fatty acids. J Nutr Metab 2012;2012:539426.
39. Serhan CN, Clish CB, Brannon J, Colgan SP, Gronert K, Chiang N. Anti-microinflammatory lipid signals generated from dietary N-3 fatty acids via cyclooxygenase-2 and transcellular processing: a novel mechanism for NSAID and N-3 PUFA therapeutic actions. J Physiol Pharmacol 2000;51(4 Pt 1):643-654.
40. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother 2002;56(8):365-379.
41. Harris WS. The omega-6/omega-3 ratio and cardiovascular disease risk: uses and abuses. Curr Atheroscler Rep 2006;8(6):453-459.
42. Hornstra G. Essential fatty acids in mothers and their neonates. Am J Clin Nutr 2000;71(5 Suppl):1262S-1269S.

Supplemental Material

Figure S3.3.1. Flow chart of the participants

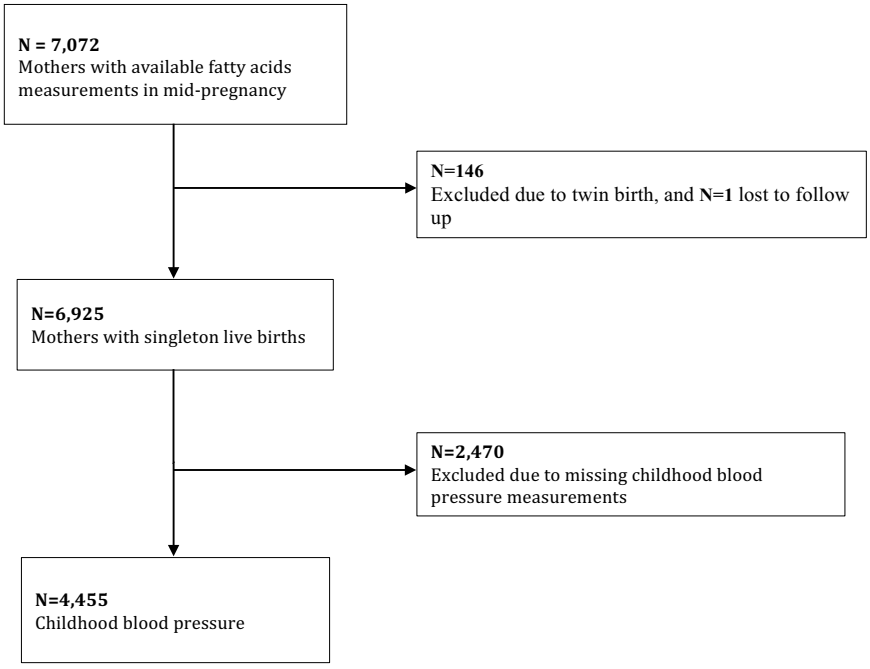


Table S3.3.1. Comparison of subject characteristics between those included and not included in the analyses (N =4,455)[1]

	Participation in follow-up N = 4,455	No participation in follow-up N = 2,470	P value
Maternal Characteristics			
Age, (yr)	30.9 (20.0, 39.2)	29.1 (18.5, 39.1)	< 0.01
Gestational age at intake, (wk)	13.8 (9.9, 22.6)	14.2 (9.8, 23.4)	< 0.01
Prepregnancy body mass index, (kg/m^2)	23.6 ± 4.2	23.7 ± 4.6	0.38
Weight gain during pregnancy, (kg)	10.4 ± 4.9	10.4 ± 5.0	0.58
Systolic blood pressure, (mmHg)	115.6 ± 12.2	114.9 ± 12.2	0.02
Diastolic blood pressure, (mmHg)	68.1 ± 9.5	67.6 ± 9.6	0.04
Education, No. higher education (%)	1938 (46.2)	764 (35.2)	< 0.01
Race / Ethnicity, No. European (%)	2683 (61.3)	1131 (50.6)	< 0.01
Parity, No. nulliparous (%)	1897 (42.9)	1117 (45.9)	0.02
Total energy intake, (kcal)	2051 ± 559	2018 ± 585	0.05
Smoking during pregnancy, No. Yes (%)	1037 (26.1)	649 (30.3)	< 0.01
Alcohol consumption during pregnancy, No. Yes (%)	2150 (54.7)	957 (45.5)	<0.01
Plasma folate, (nmol/L)	18.5 ± 9.0	18.2±10.0	0.50
Plasma homocysteine, (μmol/L)	7.1 ± 1.9	7.6±2.3	<0.01
Preeclampsia, No. (%)	80 (1.9)	66 (2.9)	< 0.01
Gestational hypertension, No. (%)	170 (4.0)	70 (3.1)	0.06
Gestational diabetes, No. (%)	40 (0.9)	30 (1.3)	0.14
Childhood characteristics			
Total PUFAs intake, (g/d)	7.9 (0.64, 38.40)	7.10 (1.9, 24.3)	0.95
N-6 PUFA intake, (g/d)	4.1 (0.03-28.89)	4.9 (0.91, 17.8)	0.48
N-3 PUFA intake, (g/d)	0.5 (0.00, 3.67)	0.5 (0.1, 2.08)	0.71
Males, No. (%)	2225 (49.9)	1274 (51.6)	0.18
Gestational age at birth, (wk)	40.1 (35.7,42.4)	40.0 (35.0, 42.3)	< 0.01
Birth weight, (g)	3436 ± 551	3387 ± 579	< 0.01
Ever breastfeeding, Yes (%)	3275 (92.8)	1289 (90.8)	< 0.01
Introduction of solid foods (%)			
< 3 months	201 (8.2)	71 (8.6)	
3-4.9 months	1484 (60.6)	522 (63.0)	0.33
>5 months	762 (31.1)	325 (28.4)	

[1]Values represent means ± SDs, median (95% range) or number of subjects (valid%).

Chapter 3.4

Maternal polyunsaturated fatty acids and childhood metabolic outcomes

Aleksandra Jelena Vidakovic, Vincent W.V. Jaddoe, Trudy Voortman, Hans Demmelmair, Berthold Koletzko, Romy Gaillard

Adapted from Nutr Metab Cardiovasc Dis. In press

Abstract

Background and Aims: Maternal polyunsaturated fatty acid (PUFA) levels are associated with cord blood lipid and insulin levels. Not much is known about the influence of maternal PUFAs during pregnancy on long-term offspring lipid and insulin metabolism. We examined the associations of maternal plasma n-3 and n-6 PUFA levels during pregnancy with childhood lipids and insulin levels.

Methods and Results: In a population-based prospective cohort study among 3,230 mothers and their children, we measured maternal second trimester n-3 and n-6 PUFA plasma levels. At the median age of 6.0 years (95% range, 5.6-7.9), we measured childhood total-cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, triglycerides, insulin and c-peptide levels. Higher maternal total n-3 PUFA levels, and specifically DHA levels, were associated with higher childhood total-cholesterol, HDL-cholesterol and insulin levels (*P* values <0.05), but not with LDL-cholesterol and triglycerides. Maternal total n-6 PUFA levels were not associated with childhood outcomes, but higher levels of the individual n-6 PUFAs, EDA and DGLA were associated with a lower childhood HDL-cholesterol, and higher AA levels with higher childhood total-cholesterol and HDL-cholesterol levels (all *P* values <0.05). A higher maternal n-6/n-3 PUFA ratio was only associated with lower childhood HDL-cholesterol and insulin levels (*P* values <0.05). These associations were not explained by childhood body mass index.

Conclusions: Higher maternal total n-3 PUFAs and specifically DHA levels during pregnancy are associated with higher childhood total-cholesterol, HDL-cholesterol, and insulin levels. Only individual maternal n-6 PUFAs, not total maternal n-6 PUFA levels, tended to be associated with childhood lipids and insulin levels.

Introduction

Suboptimal maternal nutritional status during pregnancy is associated with an increased risk of cardio-metabolic diseases in the offspring.[1] Fatty acids are required in adequate amounts for fetal growth and development.[2] During pregnancy, maternal fatty acids, mainly polyunsaturated fatty acids (PUFAs), act as precursors of eicosanoid synthesis and lipid messengers for fetal development.[3] Animal studies have shown that a maternal diet containing higher levels of PUFA during pregnancy improves the offspring's lipid profile in the postnatal period.[4,5] Among humans, studies in non-pregnant adults, showed that higher levels of docosahexaenoic acid (DHA), a n-3 PUFA, were associated with higher high-density lipoprotein (HDL)-cholesterol, lower triglycerides, and a lower risk of the metabolic syndrome, whereas higher levels of α-linolenic acid (ALA), a n-6 PUFA, seem to be associated with a higher risk of an adverse metabolic profile.[6,7] A small study among 242 Dutch mother-child pairs showed that higher levels of maternal docosapentaenoic acid (DPA), a n-3 PUFA, during pregnancy were associated with higher total-cholesterol and low-density-lipoprotein (LDL)-cholesterol levels in the offspring. In this study, higher levels of maternal linoleic acid (LA), a n-6 PUFA, were associated with higher childhood proinsulin levels.[8] However, results from randomized controlled trials that assessed the influence of fish oil supplementation during pregnancy on offspring lipids and risk of diabetes mellitus, showed inconsistent results.[9-11] Thus, the influence of different maternal n-3 and n-6 PUFAs during pregnancy on long-term offspring lipid and insulin metabolism remains unclear.[12]

Therefore, we examined, in a population-based prospective cohort study from early pregnancy onwards among 3,230 mothers and their children, the associations of maternal plasma n-3 and n-6 PUFA levels during pregnancy with childhood total-cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, insulin and c-peptide levels. We further explored whether these associations are independent of maternal and childhood socio-demographic and lifestyle-related characteristics and childhood body mass index.

Methods

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life to adulthood in Rotterdam, the Netherlands.[13] The study has been approved by the Medical Ethical Committee of Erasmus Medical Center in

Rotterdam (MEC 198.782/2001/31). All mothers gave written consent. Pregnant women with an expected delivery date from April 2002 to January 2006 were enrolled in the study. In total, 8,879 mothers were enrolled during pregnancy, of whom 7,072 had information about PUFA levels available and 6,925 gave birth to singleton live born children. Childhood lipids, insulin, or c-peptide levels were available in 3,230 of these children (a flow chart is given in **Supplemental Figure S3.4.1**). Missing blood samples were mainly due to non-consent for venous puncture or crying of the child.

Maternal fatty acid status

Maternal non-fasting venous samples were drawn at a median gestational age of 20.5 weeks (95% range: 17.1-24.9). To analyze PUFA levels, EDTA plasma samples were selected and transported to the Division of Metabolic Diseases and Nutritional Medicine, Dr. von Hauner Children's Hospital, University of Munich Medical Center. After being thawed, the analysis of plasma glycerophospholipid PUFA composition was performed using gas chromatography by a sensitive and precise high-throughput method, suitable for large epidemiological studies, as previously described.[14] Based on findings from previous studies, for our analyses we selected maternal PUFA that have been associated with the risk of cardiovascular and metabolic outcomes in adults, and with pregnancy and fetal outcomes.[8,15] Selected total maternal PUFA were total n-3 PUFA, which included: α-linolenic acid (ALA, C18:3n3), eicosapentaenoic acid (EPA, C20:5n3), docosapentaenoic acid (DPA, C22:5n-3) and docosahexaenoic acid (DHA, C22:6n3). Total n-6 PUFA included: linoleic acid (LA, C18:2n6), γ-linolenic acid (GLA, C18:3n-6), eicosadienoic acid (EDA, C20:2n-6), dihomo-γ-linolenic acid (DGLA, C20:3n6), arachidonic acid (AA, C20:4n6) and docosatetraenoic acid (DTA, C22:4n-6). PUFA levels were expressed as proportion of total fatty acids present in the chromatogram (weight percentage, wt%) to express the relative importance of a fatty acids set against the total fatty acids concentrations.[16] We also calculated the ratio of total n-6/n-3 PUFA.

Childhood lipids and insulin measurements

At the age of 6 years (95% range, 5.6-7.9), total-cholesterol, HDL-cholesterol, and LDL-cholesterol, triglycerides, insulin and c-peptide levels were obtained enzymatically from venous blood samples 30 minutes after the last meal using a Cobas 8000 analyzer (Roche, Almere, The Netherlands).[17] Quality control samples demonstrated intra- and interassay coefficients of variation ranging from 0.77 to 1.39%, and 0.87 to 2.40%, respectively.

Covariates

Information on maternal age, educational level, ethnicity, and parity, was obtained with questionnaires at enrolment.[13] We calculated body mass index. Information on maternal smoking habits during pregnancy and folic acid supplement use was assessed by self-reported questionnaires during pregnancy. Weight gain up to 30 weeks of gestation was calculated as the difference between maternal weight measured at 30 weeks of gestation and self-reported weight before pregnancy, as described previously.[18] We used a 293-item food frequency questionnaire covering the first trimester to assess maternal dietary intake during pregnancy. Information about pregnancy complications, child's sex, gestational age at birth, and weight at birth was obtained from medical records.[19,20] Information about breastfeeding, timing of introduction of solid foods and average TV watching time was obtained by questionnaires in infancy.[21] Information about infant PUFA intake at 13 months, measured with a 211-item food frequency questionnaire was available in a subgroup of the study (n = 1,566).[22]

Statistical analysis

We used linear regression analyses to assess the associations of maternal total and individual plasma n-3 and n-6 PUFA levels with the childhood outcomes. The regression models were adjusted for maternal age, educational level, ethnicity, parity, prepregnancy body mass index, smoking habits during pregnancy, folic acid supplement use, total caloric intake during pregnancy, pregnancy complications, child's sex, gestational age-adjusted birth weight standard-deviation scores (SDS), breastfeeding duration, timing of introduction of solid foods, and TV watching time. We additionally adjusted these analyses for children's body mass index at the age of 6 years to explore if these associations were explained by childhood body mass index. For all analyses, we log-transformed not normally distributed childhood outcomes (triglycerides and insulin). We constructed SDS [(observed value - mean)/ SD] for all PUFAs and childhood outcomes measures to enable comparison of effect estimates. We tested for interaction terms between maternal PUFA levels and child's sex in relation to the childhood outcomes, but since no significant interactions were present no further stratified analyses were performed. In order to reduce potential bias associated with missing data and to maintain statistical power, we performed multiple imputations of missing covariates by generating 5 independent datasets using the Markov Chain Monte Carlo method after which the pooled effect estimates were calculated. All analyses were performed using Statistical Package for the Social Sciences (SPSS) version 21.0 for Windows (IBM Corp, Chicago, IL, Armonk, NY, USA).

Results

Subject characteristics

Table 3.4.1 shows the maternal and childhood characteristics. Mean (SD) second trimester maternal plasma levels of n-3 and n-6 PUFA were 105.4 mg/L (27.7) and 602.8 mg/L (89.0), respectively **(Table 3.4.2)**. Results from the non-response analysis are given in **Supplemental Table S3.4.1** and show that mothers who were not included in the analyses smoked more often during pregnancy and developed more often gestational hypertensive disorders as compared to those who were included (p-values<0.05). Mothers not included in the analyses had lower total n-3 PUFA levels compared to those included (**Supplemental Table S3.4.2**). Correlation coefficients between all maternal PUFA levels are shown in **Supplemental Table S3.4.3.**

Table 3.4.1. Characteristics of mothers and their children (N =3,230)[a]

	Value
Maternal characteristics	
Age, (y), median (95% range)	31.0 (20.1, 39.4)
Gestational age at fatty acid measures, (weeks), median (95% range)	20.5 (17.1, 24.9)
Pre-pregnancy body mass index, (kg/m2), mean (SD)	23.6 (4.2)
Weight gain, (kg), mean (SD)	10.4 (4.9)
Education, No. higher education (%)	1455 (47.7)
Ethnicity, No. European (%)	1963 (61.9)
Parity, No. nulliparous (%)	1808 (56.3)
Smoking during pregnancy, No. yes (%)	736 (25.6)
Folic acid supplement use, No. yes (%)	1867 (75.7)
Gestational diabetes, No. (%)	27 (0.9)
Gestational hypertensive disorders, No. (%)	176 (5.6)
Birth and infant characteristics	
Males, No. (%)	1650 (51.1)
Gestational age at birth (weeks), median (95% range)	40.1 (35.8, 42.3)
Breastfeeding duration (months), mean (SD)	4.5 (3.9)
Introduction of solid foods No. (%) >6 months	208 (10.1)
N-3 PUFA intake (g/d)	0.6 (0.4)
N-6 PUFA intake (g/d)	4.7 (3.0)
Childhood outcomes at 6 years	
Body mass index at 6 years (kg/m^2), mean (SD)	16.2 (1.8)
TV watching time, No. (%)	
< 2 hours/day	2059 (80.6)
≥ 2 hours/day	496 (19.4)
Total-cholesterol (mmol/L), mean (SD)	4.2 (0.6)
HDL-cholesterol (mmol/L), mean (SD)	1.3 (0.3)
LDL-cholesterol (mmol/L), mean (SD)	2.4 (0.6)
Triglycerides (mmol/L), median (95% range)	1.0 (0.4-2.5)
Insulin (pmol/L), median (95% range)	141 (17-421)
C-peptide (ng/mL), median (95% range)	1.0 (0.3-2.1)

[a]Values represent means ± SDs, median (95% range) or number of subjects (valid%).

Table 3.4.2. Second trimester maternal PUFA levels in plasma (N =3,230)[a]

	Absolute values (mg/L)	Relative values (wt%)
Total PUFA	708.3 ± 98.8	43.1 ± 2.0
Total n-3 PUFA	105.4 ± 27.7	6.5 ± 1.5
ALA	5.1 ± 1.8	0.3 ± 0.1
EPA	8.8 ± 5.5	0.5 ± 0.3
DPA	12.1 ± 4.4	0.7 ± 0.2
DHA	77.9 ± 20.5	4.8 ± 1.1
Total n-6 PUFA	602.8 ± 89.0	37.2 ± 2.5
LA	361.1 ± 63.5	22.3 ± 2.8
GLA	1.5 ± 0.7	0.1 ± 0.1
EDA	8.5 ± 1.9	0.5 ± 0.1
DGLA	61.0 ± 16.5	3.7 ± 0.7
AA	156.4 ± 32.6	9.6 ± 1.6
DTA	6.9 ± 2.2	0.4 ± 1.1
Total n-6/n-3 PUFAs ratio	6.1 ± 1.7	-

[a]Values represent means ± SDs.

Abbreviations: AA: arachidonic acid; ALA: α-linolenic acid; DGLA dihomo-γ-linolenic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; DTA: docosatetraenoic acid; EDA: eicosadienoic acid; EPA: eicosapentaenoic acid; GLA: γ-linolenic acid; LA: linoleic acid; PUFA: polyunsaturated fatty acid.

Maternal n-3 PUFAs with childhood lipids and insulin levels

Table 3.4.3 shows that, after adjustment for potential confounders, higher maternal total n-3 PUFA levels were associated with higher childhood total-cholesterol, HDL-cholesterol and insulin levels (differences: 0.04 (95% CI:0.01, 0.08), 0.06 (95% CI: 0.02, 0.09) and 0.05 (95% CI: 0.01, 0.08, respectively) per SD increase of maternal total n-3 PUFA levels), but not with childhood LDL-cholesterol and triglycerides. Among the individual n-3 PUFA levels, we observed that higher maternal EPA levels were only associated with a higher childhood HDL-cholesterol, whereas higher levels of maternal DPA were only associated with higher childhood insulin levels (all p-values<0.05). Higher maternal DHA levels were associated with higher childhood total-cholesterol, HDL-cholesterol and insulin levels (differences: 0.04 (95% CI: 0.01, 0.08), 0.06 (95%CI: 0.02, 0.09) and 0.04 (95% CI: 0.01, 0.08, respectively) per SD increase of maternal DHA levels), but not with childhood LDL-cholesterol and triglycerides. We observed similar results when we used childhood c-peptide levels instead of childhood insulin levels (**Supplemental Tables S3.4.4**). Results did not materially change after additional adjustment for childhood concurrent body mass index.

Table 3.4.3. Maternal plasma n-3 PUFA levels and childhood lipids and insulin levels (N=3,230)[a]

Maternal n-3 PUFA in SDS	Differences in childhood lipid and insulin outcomes (95% confidence interval) in SDS				
	Total-cholesterol	HDL-cholesterol	LDL-cholesterol	Triglycerides	Insulin
Total n-3 PUFA					
Adjusted model[b]	0.04 (0.01, 0.08)*	0.06 (0.02, 0.09)*	0.02 (-0.02, 0.05)	-0.03 (-0.07, 0.01)	0.05 (0.01, 0.08)*
Childhood BMI model[c]	0.04 (0.01, 0.08)*	0.06 (0.02, 0.09)*	0.02 (-0.02, 0.05)	-0.03 (-0.07, 0.01)	0.05 (0.01, 0.08)*
ALA					
Adjusted model	-0.02 (-0.06, 0.02)	-0.02 (-0.06, 0.01)	-0.03 (-0.07, 0.01)	0.01 (-0.02, 0.05)	-0.01 (-0.05, 0.03)
Childhood BMI model	-0.02 (-0.06, 0.02)	-0.03 (-0.06, 0.01)	-0.03 (-0.07, 0.01)	0.01 (-0.02, 0.05)	-0.01 (-0.04, 0.03)
EPA					
Adjusted model	0.03 (-0.01, 0.07)	0.07 (0.03, 0.10)*	0.01 (-0.03, 0.04)	-0.03 (-0.07, 0.01)	0.04 (-0.01, 0.07)
Childhood BMI model	0.03 (-0.01, 0.07)	0.07 (0.03, 0.10)*	0.01 (-0.03, 0.04)	-0.03 (-0.07, 0.01)	0.04 (-0.01, 0.07)
DPA					
Adjusted model	0.01 (-0.03, 0.04)	0.01 (-0.03, 0.04)	-0.01 (-0.04, 0.04)	-0.01 (-0.05, 0.02)	0.04 (0, 0.07)*
Childhood BMI model	0.01 (-0.03, 0.04)	0.01 (-0.03, 0.04)	0.01 (-0.03, 0.04)	-0.01 (-0.05, 0.02)	0.04 (0.01, 0.08)*
DHA					
Adjusted model	0.04 (0.01, 0.08)*	0.06 (0.02, 0.09)*	0.02 (-0.02, 0.06)	-0.03 (-0.06, 0.01)	0.04 (0.01, 0.08)
Childhood BMI model	0.04 (0.01, 0.08)*	0.06 (0.02, 0.09)*	0.02 (-0.02, 0.06)	-0.03 (-0.06, 0.01)	0.04 (0.01, 0.08)*

[a]Values are regression coefficients (95% CIs) that reflect the difference in SDS of childhood lipids and insulin levels per SD change in maternal n-3 PUFA levels, respectively. [b]Adjusted model is adjusted for maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, smoking habits during pregnancy, folic acid supplement use and total caloric intake during pregnancy and pregnancy complications, and child's sex, gestational age-adjusted birth weight standard-deviation scores, breastfeeding duration, timing of introduction of solid foods, and TV watching time. [c]Childhood BMI model is additionally adjusted for body mass index standard-deviation scores at 6 years. *P-value<0.05. Abbreviations: ALA: α-linolenic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; PUFA; polyunsaturated fatty acid.

Maternal n-6 PUFAs with childhood lipids and insulin levels

Table 3.4.4 shows that, after adjustment for potential confounders, higher maternal total n-6 PUFA, LA and GLA levels were not associated with any of the childhood lipid and insulin outcomes. Higher maternal EDA and DGLA levels were only associated with a lower childhood HDL-cholesterol (p-value<0.05). Higher maternal AA levels were associated with higher childhood total-cholesterol and HDL-cholesterol levels (differences: 0.04 (95% CI: 0.01, 0.08) and 0.05 (95% CI: 0.01, 0.08, respectively) per SD increase of maternal AA levels), but not with childhood LDL-cholesterol, triglycerides or insulin levels. We observed similar results when we used childhood c-peptide levels instead of childhood insulin levels (**Supplemental Tables S3.4.5**). These results were not materially affected by additional adjustment for childhood body mass index.

Table 3.4.4. Maternal plasma n-6 PUFA levels and childhood lipids and insulin levels (N=3,230)[a]

Maternal n-6 PUFA in SDS	Differences in childhood lipid and insulin outcomes (95% confidence interval) in SDS				
	Total-cholesterol	HDL-cholesterol	LDL-cholesterol	Triglycerides	Insulin
Total n-6 PUFA					
Adjusted model[2]	0.01 (-0.03, 0.05)	0.01 (-0.03, 0.05)	0.02 (-0.02, 0.06)	0.02 (-0.02, 0.06)	-0.02 (-0.06, 0.02)
Childhood BMI model[3]	0.01 (-0.03, 0.05)	0.01 (-0.03, 0.05)	0.01 (-0.03, 0.05)	0.02 (-0.02, 0.06)	-0.02 (0.06, 0.02)
LA					
Adjusted model	-0.01 (-0.05, 0.02)	0.01 (-0.03, 0.04)	-0.01 (-0.04, 0.03)	0.01 (-0.03, 0.05)	-0.02 (-0.05, 0.02)
Childhood BMI model	-0.01 (-0.05, 0.03)	0.01 (-0.03, 0.04)	-0.01 (-0.04, 0.03)	0.01 (-0.03, 0.05)	-0.01 (-0.05, 0.02)
GLA					
Adjusted model	-0.02 (-0.06, 0.02)	-0.02 (-0.06, 0.01)	-0.02 (-0.06, 0.02)	0.01 (-0.03, 0.04)	0.01 (-0.03, 0.04)
Childhood BMI model	-0.02 (-0.05, 0.02)	-0.02 (-0.06, 0.01)	-0.02 (-0.05, 0.02)	0.01 (-0.03, 0.04)	0.01 (-0.03, 0.04)
EDA					
Adjusted model	-0.02 (-0.06, 0.01)	-0.04 (-0.07, -0.01)*	-0.01 (-0.05, 0.02)	0.01 (-0.03, 0.05)	-0.02 (-0.05, 0.02)
Childhood BMI model	-0.02 (-0.06, 0.01)	-0.04 (-0.07, -0.01)*	-0.01 (-0.05, 0.02)	0.01 (-0.03, 0.04)	-0.02 (-0.05, 0.02)
DGLA					
Adjusted model	-0.01 (-0.05, 0.03)	-0.07 (-0.10, -0.03)*	0.01 (-0.04, 0.04)	0.06 (0.02, 0.10)*	-0.03 (-0.06, 0.01)
Childhood BMI model	-0.01 (-0.05, 0.03)	-0.07 (-0.10, -0.03)*	0.01 (-0.04, 0.04)	0.06 (0.02, 0.09)*	-0.03 (-0.06, 0.01)
AA					
Adjusted model	0.04 (0.01, 0.08)*	0.05 (0.01, 0.08)*	0.03 (-0.01, 0.07)	-0.02 (-0.06, 0.02)	0.02 (-0.01, 0.06)
Childhood BMI model	0.04 (0.01, 0.07)*	0.05 (0.01, 0.09)*	0.03 (-0.01, 0.06)	-0.02 (-0.06, 0.01)	0.02 (-0.02, 0.05)
DTA					
Adjusted model	-0.02 (-0.06, 0.02)	-0.03 (-0.07, 0.01)	0.01 (-0.03, 0.04)	-0.01 (-0.04, 0.03)	-0.02 (-0.05, 0.02)
Childhood BMI model	-0.02 (-0.06, 0.02)	-0.03 (-0.07, 0.01)	0.01 (-0.03, 0.04)	-0.01 (-0.04, 0.03)	-0.02 (-0.06, 0.02)

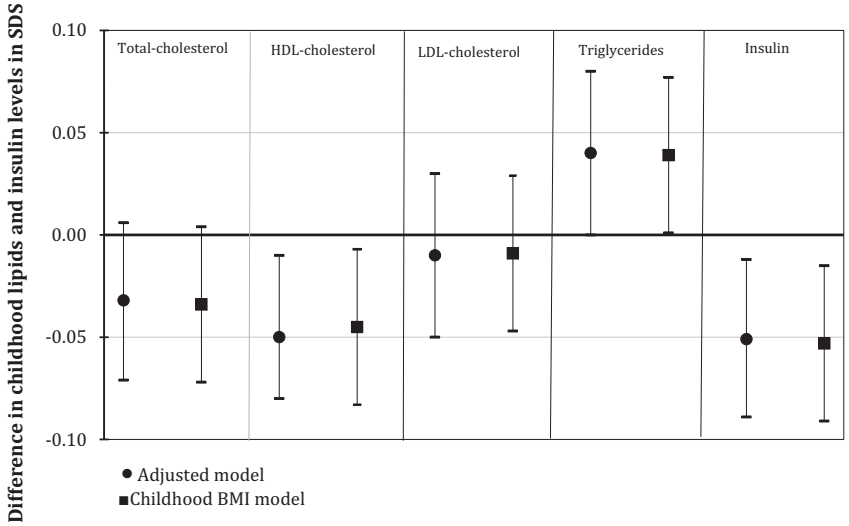
[a]Values are regression coefficients (95% CIs) that reflect the difference in SDS of childhood lipids and insulin levels per SD change in maternal n-6 PUFA levels, respectively. [b]Adjusted model is adjusted for maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, smoking habits during pregnancy, folic acid supplement use and total caloric intake during pregnancy and pregnancy complications, and child's sex, gestational age-adjusted birth weight standard-deviation scores, breastfeeding duration, timing of introduction of solid foods, and TV watching time. [c]Childhood BMI model is additionally adjusted for body mass index standard-deviation scores at 6 years. *P-value<0.05.

Abbreviations: AA: arachidonic acid; DGLA dihomo-gamma-linolenic acid; DGLA dihomo-γ-linolenic acid; DTA: docosatetraenoic acid; EDA: eicosadienoic acid; GLA: γ-linolenic acid; LA: linoleic acid; PUFA; polyunsaturated fatty acid.

Maternal n-6/n-3 PUFAs ratio with childhood lipids and insulin levels

Figure 3.4.1 shows that a higher maternal n-6/n-3 PUFA ratio was not associated with childhood total-cholesterol and LDL-cholesterol levels. A higher maternal n-6/n-3 PUFA ratio was associated with lower childhood HDL-cholesterol and insulin levels (differences: -0.05 (95% CI:-0.08, -0.01) and -0.05 (95% CI: -0.09, -0.01) per SD increase of maternal n-6/n-3 PUFA ratio), but with higher childhood triglycerides levels (difference: 0.04 (95% CI: 0, 0.08) per SD increase of maternal n-6/n-3 PUFA ratio). These associations were not explained by additional adjustment for childhood body mass index. Additional adjustment for infants' PUFA intakes at 13 months did

Figure 3.4.1. Maternal plasma n-6/n-3 PUFA ratio and childhood lipids and insulin levels (N =3,320)



Values are regression coefficients (95% CIs) that reflect the difference in SDS of childhood lipids and insulin levels per SD change in maternal n-6/n-3 PUFAs ratio, respectively. Models are adjusted for gestational age at blood sampling, maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, smoking habits during pregnancy, folic acid supplement use and total caloric intake during pregnancy and pregnancy complications, and child's sex, gestational age-adjusted birth weight standard-deviation scores, breastfeeding duration, timing of introduction of solid foods, and TV watching time. *P-value<0.05.

not affect the observed associations (data not shown). Replacing child body mass index by fat mass index did not change the results (results not shown).

Discussion

We observed that higher maternal total n-3 PUFA and specifically DHA levels during pregnancy were associated with higher childhood total-cholesterol, HDL-cholesterol, and insulin levels. The individual maternal n-6 PUFAs, EDA, DGLA and AA, but not total maternal n-6 PUFA levels during pregnancy, were associated with childhood total and HDL-cholesterol levels in different directions. These associations were not explained by childhood concurrent body mass index.

Methodological considerations

We used a population-based prospective cohort study design with a large number of subjects. Of all children whose maternal PUFA levels were available, 64% participated in the follow-up studies at the age of 6 years. The non-response could lead to biased effect estimates if the associations of maternal PUFA levels with childhood lipids and

insulin levels would be different between children included and not included in the analyses. Non-response analysis showed that mothers included in the analyses had higher total n-3 PUFA levels and a lower n-6/n-3 PUFAs ratio compared to those not included. It is hard to speculate whether these differences would have affected the observed associations materially. We measured a large number of maternal PUFA levels in plasma samples once during pregnancy. No information was available about PUFA levels earlier or later in pregnancy. Nevertheless, PUFAs measured in plasma reflect a time frame of dietary intake of approximately 2 weeks and seem to be reasonable indicators for the recent intake.[23] The fasting time before venous punctures was limited. Due to the young age of the children and the structure of the follow-up visits, it was ethically not possible to obtain 3-hours fasting blood samples. We used blood samples obtained 30-minutes after the last meal to measure childhood lipids, insulin and c-peptide levels. This may have led to non-differential misclassification and an underestimation of the associations with maternal PUFAs. It has only been shown among adult populations that also non-fasting lipid and insulin levels are associated with increased risks of cardiovascular events.[24-26] Further studies are needed with more detailed fasting measurements of offspring lipids and insulin metabolism to assess the associations of maternal PUFA status during pregnancy with both fasting and non-fasting offspring lipid and insulin metabolism throughout the life course. The percentages of women with gestational diabetes were relatively low within our study cohort. Accurate diagnosis of gestational diabetes is difficult. Information on gestational diabetes was obtained from the medical records. Unfortunately, in our study, no data were available on glucose tolerance before and during pregnancy, which would have allowed a better identification of women at risk of development of gestational diabetes. The prevalence in our study was lower than those in previous studies, which may be explained by use of different criteria for gestational diabetes and the relatively healthy study population within our study cohort.[27] Finally, although we performed adjustment for a large number of potential maternal and childhood confounders, residual confounding might still occur, as in any observational study.

Interpretation of main findings

Suboptimal maternal nutritional status during pregnancy is associated with an increased risk of cardio-metabolic diseases in the offspring.[28,29] Previous studies have shown that maternal PUFA status during pregnancy can affect lipid and insulin metabolism, measured in cord blood.[8,30,31] However, associations with lipid and insulin metabolism in the offspring at later age are less clear.

Previous studies on different maternal PUFAs during pregnancy and offspring lipid metabolism have shown inconsistent results. A study among 965 Danish pregnant women reported no associations between dietary intake of total n-3 PUFA during the second trimester of pregnancy and total-cholesterol, HDL-cholesterol and LDL-cholesterol levels in the 19-year-old offspring.[32] In the same study, offspring triglyceride levels were higher among children whose mothers had total n-3 PUFA intake in the higher quintiles as compared to the lowest quintile. A study among 243 mothers and their offspring from Denmark showed no effect of fish oil supplementation during the third trimester of pregnancy on plasma total-cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, and lipoproteins in their 19-years-old offspring.[9] A small study in 242 mother-child pairs from the Netherlands showed that a higher maternal DPA, a n-3 PUFA, measured at the first trimester of pregnancy was associated with higher total-cholesterol and LDL-cholesterol levels in 7-year-old children. In the same study, a higher maternal AA level, a n-6 PUFA, was associated with lower childhood triglycerides and LDL-cholesterol levels, but no associations were observed for other n-6 PUFA levels.[8] In this current study, we observed that higher maternal total n-3 PUFA and DHA levels during pregnancy were associated with higher childhood total-cholesterol and HDL-cholesterol levels. No associations of maternal total n-6 PUFAs during pregnancy with childhood lipids were present. However, among the individual n-6 PUFAs, higher maternal EDA and DGLA levels were associated with lower childhood HDL-cholesterol, whereas higher AA levels were associated with higher childhood total-cholesterol and HDL-cholesterol levels. In addition, a higher maternal n-6/n-3 PUFA ratio was associated with a lower HDL-cholesterol, but with higher childhood triglycerides levels. These associations were independent of childhood body mass index. Thus, our results suggest that a suboptimal maternal PUFA status during pregnancy is associated with childhood lipid levels, independent of childhood current body mass index. It appears that individual maternal n-3 and n-6 PUFAs may have alternating effects on offspring lipids metabolism. DHA and AA are preferentially transferred from the maternal to the fetal circulation by means of a preferential placental transfer mediated by fatty acid transport and binding proteins[33,34] which may partly explain the observed associations specifically for these PUFAs since they comprise a relative large part of the total fetal PUFA pool.

Previous studies assessing the influence of maternal PUFAs intake on offspring insulin sensitivity also reported inconsistent results.[10,11,31] A randomized controlled trial among 533 pregnant Danish women showed no effect of n-3 PUFA supplementation during the third trimester of pregnancy on insulin levels in 19-year-old offspring.[10] A case-control study among 85 diabetic mothers and 1071 controls from Norway reported that cod liver oil supplementation, a n-3 PUFA, during pregnancy

was associated with reduced risk of offspring type I diabetes mellitus.[11] Another trial among 47 women and their infants showed that mothers consuming DHA, a n-3 PUFA, during the last half of pregnancy had lower cord blood insulin levels, compared to the control group.[31] A study among 242 Dutch mother-child pairs showed that higher levels of EPA, a n-3 PUFA, were associated with lower glucose levels in 7-year-old children, whereas only higher levels of maternal LA, a n-6 PUFA, were associated with children's higher proinsulin levels.[8] Contrary to these previous studies, we observed that higher maternal total n-3 PUFA and specifically DHA levels during pregnancy were associated with higher childhood insulin and c-peptide levels, independent of childhood concurrent body mass index. No associations were observed between maternal n-6 PUFA levels and childhood insulin levels. Differences between our study and previous studies may be explained by differences in study design, age of participants and different methods to assess maternal PUFA status during pregnancy and childhood lipid and insulin/ glucose metabolism.[8-11,31,32] The previous studies all used fasting blood samples, whereas in our study we used non-fasting blood samples to measure childhood lipid and insulin metabolism.[8-10,32] Thus far, it remains unclear whether maternal PUFA status has different effects on offspring fasting vs non-fasting lipid and insulin metabolism. Further studies are needed to assess the detailed associations of maternal PUFA levels during pregnancy with offspring fasting and non-fasting measures of lipid and insulin metabolism.

The mechanisms underlying these observed associations are not known. We did not observe an effect of additional adjustment for childhood body mass index, which suggests that these associations may not be explained by the influence of childhood adiposity on lipid and insulin metabolism. Maternal n-3 PUFAs may affect offspring total-cholesterol and triglycerides through a reduction of hepatic synthesis of triacylglycerols and very-low-density-lipoprotein.[35] Furthermore, as a part of the cell membrane, n-3 PUFAs may be able to regulate insulin secretion from pancreatic b-cells directly by altering lipid raft structure.[36] Among n-6 PUFAs, AA may play a beneficial metabolic role in the health of offspring together with EPA and DHA during critical periods of fetal development through changes in gene expression, the production of eicosanoids and inflammatory markers.[37,38] PUFAs may also affect offspring insulin levels during pregnancy through epigenetic regulation of imprinted genes, especially insulin growth factor 2 (IGF2), which is known to control fetal growth, development and insulin/glucose metabolism.[39] Future research focused on epigenetic mechanisms is needed to assess the effect of PUFAs during pregnancy on epigenetic regulation of imprinted genes, including IGF2, which may be involved in the underlying mechanisms in the observed associations.

Conclusion

We observed that higher maternal total n-3 PUFAs and specifically DHA levels during pregnancy were associated with higher childhood total-cholesterol, HDL-cholesterol, and insulin levels. Only specific individual maternal n-6 PUFAs during pregnancy, not total maternal n-6 PUFA levels, tended to be associated with childhood lipids and insulin levels. Further observational and experimental studies are needed for replication of our findings and to obtain further insight into the potential different role of individual maternal PUFAs on offspring lipid and insulin metabolism.

References

1. Henriksen T, Clausen T. The fetal origins hypothesis: placental insufficiency and inheritance versus maternal malnutrition in well-nourished populations. Acta Obstet Gynecol Scand 2002;81(2):112-114.
2. Haggarty P. Fatty acid supply to the human fetus. Annu Rev Nutr 2010;30:237-255.
3. Innis SM. Essential fatty acid transfer and fetal development. Placenta 2005;26 Suppl A:S70-75.
4. Bremer AA, Stanhope KL, Graham JL, Cummings BP, Ampah SB, Saville BR, Havel PJ. Fish oil supplementation ameliorates fructose-induced hypertriglyceridemia and insulin resistance in adult male rhesus macaques. J Nutr 2014;144(1):5-11.
5. Chechi K, Cheema SK. Maternal diet rich in saturated fats has deleterious effects on plasma lipids of mice. Exp Clin Cardiol 2006;11(2):129-135.
6. Zhang G, Sun Q, Hu FB, Ye X, Yu Z, Zong G, Li H, Zhou Y, Lin X. Erythrocyte n-3 fatty acids and metabolic syndrome in middle-aged and older Chinese. J Clin Endocrinol Metab 2012;97(6):E973-977.
7. Huang T, Bhulaidok S, Cai Z, Xu T, Xu F, Wahlqvist ML, Li D. Plasma phospholipids n-3 polyunsaturated fatty acid is associated with metabolic syndrome. Mol Nutr Food Res 2010;54(11):1628-1635.
8. Jochems SH, Gielen M, Rump P, Hornstra G, Zeegers MP. Potential programming of selected cardiometabolic risk factors at childhood by maternal polyunsaturated fatty acid availability in the MEFAB cohort. Prostaglandins Leukot Essent Fatty Acids 2015;100:21-27.
9. Rytter D, Schmidt EB, Bech BH, Christensen JH, Henriksen TB, Olsen SF. Fish oil supplementation during late pregnancy does not influence plasma lipids or lipoprotein levels in young adult offspring. Lipids 2011;46(12):1091-1099.
10. Rytter D, Bech BH, Christensen JH, Schmidt EB, Henriksen TB, Olsen SF. Intake of fish oil during pregnancy and adiposity in 19-y-old offspring: follow-up on a randomized controlled trial. Am J Clin Nutr 2011;94(3):701-708.
11. Stene LC, Ulriksen J, Magnus P, Joner G. Use of cod liver oil during pregnancy associated with lower risk of Type I diabetes in the offspring. Diabetologia 2000;43(9):1093-1098.
12. Voortman T, van den Hooven EH, Braun KV, van den Broek M, Bramer WM, Chowdhurry R, Franco OH. Effects of polyunsaturated fatty acid intake and status during pregnancy, lactation, and early childhood on cardiometabolic health: A systematic review. Prog Lipid Res 2015;59:67-87.
13. Jaddoe VW, van Duijn CM, Franco OH, van der Heijden AJ, van Iizendoorn MH, de Jongste JC, van der Lugt A, Mackenbach JP, Moll HA, Raat H, Rivadeneira F, Steegers EA, Tiemeier H, Uitterlinden AG,

Verhulst FC, Hofman A. The Generation R Study: design and cohort update 2012. Eur J Epidemiol 2012;27(9):739-756.

14. Glaser C, Demmelmair H, Koletzko B. High-throughput analysis of fatty acid composition of plasma glycerophospholipids. J Lipid Res 2010;51(1):216-221.
15. Wijendran V, Bendel RB, Couch SC, Philipson EH, Thomsen K, Zhang X, Lammi-Keefe CJ. Maternal plasma phospholipid polyunsaturated fatty acids in pregnancy with and without gestational diabetes mellitus: relations with maternal factors. Am J Clin Nutr 1999;70(1):53-61.
16. Mocking RJ, Assies J, Lok A, Ruhe HG, Koeter MW, Visser I, Bockting CL, Schene AH. Statistical methodological issues in handling of fatty acid data: percentage or concentration, imputation and indices. Lipids 2012;47(5):541-547.
17. Kruithof CJ, Kooijman MN, van Duijn CM, Franco OH, de Jongste JC, Klaver CC, Mackenbach JP, Moll HA, Raat H, Rings EH, Rivadeneira F, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC, Wolvius EB, Hofman A, Jaddoe VW. The Generation R Study: Biobank update 2015. Eur J Epidemiol 2014;29(12):911-927.
18. Gaillard R, Durmus B, Hofman A, Mackenbach JP, Steegers EA, Jaddoe VW. Risk factors and outcomes of maternal obesity and excessive weight gain during pregnancy. Obesity (Silver Spring) 2013;21(5):1046-1055.
19. Verburg BO, Steegers EA, De Ridder M, Snijders RJ, Smith E, Hofman A, Moll HA, Jaddoe VW, Witteman JC. New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. Ultrasound Obstet Gynecol 2008;31(4):388-396.
20. Niklasson A, Ericson A, Fryer JG, Karlberg J, Lawrence C, Karlberg P. An update of the Swedish reference standards for weight, length and head circumference at birth for given gestational age (1977-1981). Acta Paediatr Scand 1991;80(8-9):756-762.
21. Heppe DH, Medina-Gomez C, Hofman A, Franco OH, Rivadeneira F, Jaddoe VW. Maternal first-trimester diet and childhood bone mass: the Generation R Study. Am J Clin Nutr 2013;98(1):224-232.
22. Voortman T, Kiefte-de Jong JC, Geelen A, Villamor E, Moll HA, de Jongste JC, Raat H, Hofman A, Jaddoe VW, Franco OH, van den Hooven EH. The development of a diet quality score for preschool children and its validation and determinants in the Generation R Study. J Nutr 2015;145(2):306-314.
23. Skeaff CM, Hodson L, McKenzie JE. Dietary-induced changes in fatty acid composition of human plasma, platelet, and erythrocyte lipids follow a similar time course. J Nutr 2006;136(3):565-569.
24. Bansal S, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. JAMA 2007;298(3):309-316.
25. Sarwar N, Sattar N, Gudnason V, Danesh J. Circulating concentrations of insulin markers and coronary heart disease: a quantitative review of 19 Western prospective studies. Eur Heart J 2007;28(20):2491-2497.
26. Langsted A, Freiberg JJ, Nordestgaard BG. Fasting and nonfasting lipid levels: influence of normal food intake on lipids, lipoproteins, apolipoproteins, and cardiovascular risk prediction. Circulation 2008;118(20):2047-2056.
27. Sacks DA, Hadden DR, Maresh M, Deerochanawong C, Dyer AR, Metzger BE, Lowe LP, Coustan DR, Hod M, Oats JJ, Persson B, Trimble ER, Group HSCR. Frequency of gestational diabetes mellitus at collaborating centers based on IADPSG consensus panel-recommended criteria: the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study. Diabetes Care 2012;35(3):526-528.
28. Godfrey KM, Barker DJ. Fetal nutrition and adult disease. Am J Clin Nutr 2000;71(5 Suppl):1344S-1352S.

29. Armitage JA, Khan IY, Taylor PD, Nathanielsz PW, Poston L. Developmental programming of the metabolic syndrome by maternal nutritional imbalance: how strong is the evidence from experimental models in mammals? J Physiol 2004;561(Pt 2):355-377.
30. Zhao JP, Levy E, Fraser WD, Julien P, Delvin E, Montoudis A, Spahis S, Garofalo C, Nuyt AM, Luo ZC. Circulating docosahexaenoic acid levels are associated with fetal insulin sensitivity. PLoS One 2014;9(1):e85054.
31. Courville AB, Harel O, Lammi-Keefe CJ. Consumption of a DHA-containing functional food during pregnancy is associated with lower infant ponderal index and cord plasma insulin concentration. Br J Nutr 2011;106(2):208-212.
32. Rytter D, Bech BH, Halldorsson T, Christensen JH, Schmidt EB, Danielsen I, Henriksen TB, Olsen SF. No association between the intake of marine n-3 PUFA during the second trimester of pregnancy and factors associated with cardiometabolic risk in the 20-year-old offspring. Br J Nutr 2013;110(11):2037-2046.
33. Larque E, Demmelmair H, Gil-Sanchez A, Prieto-Sanchez MT, Blanco JE, Pagan A, Faber FL, Zamora S, Parrilla JJ, Koletzko B. Placental transfer of fatty acids and fetal implications. Am J Clin Nutr 2011;94(6 Suppl):1908S-1913S.
34. Prieto-Sanchez MT, Ruiz-Palacios M, Blanco-Carnero JE, Pagan A, Hellmuth C, Uhl O, Peissner W, Ruiz-Alcaraz AJ, Parrilla JJ, Koletzko B, Larque E. Placental MFSD2a transporter is related to decreased DHA in cord blood of women with treated gestational diabetes. Clin Nutr 2016.
35. Mennitti LV, Oliveira JL, Morais CA, Estadella D, Oyama LM, Oller do Nascimento CM, Pisani LP. Type of fatty acids in maternal diets during pregnancy and/or lactation and metabolic consequences of the offspring. J Nutr Biochem 2015;26(2):99-111.
36. Wang X, Chan CB. n-3 polyunsaturated fatty acids and insulin secretion. J Endocrinol 2015;224(3):R97-106.
37. Calder PC. Polyunsaturated fatty acids, inflammation, and immunity. Lipids 2001;36(9):1007-1024.
38. Madsen L, Pedersen LM, Liaset B, Ma T, Petersen RK, van den Berg S, Pan J, Muller-Decker K, Dulsner ED, Kleemann R, Kooistra T, Doskeland SO, Kristiansen K. cAMP-dependent signaling regulates the adipogenic effect of n-6 polyunsaturated fatty acids. J Biol Chem 2008;283(11):7196-7205.
39. Lee HS, Barraza-Villarreal A, Biessy C, Duarte-Salles T, Sly PD, Ramakrishnan U, Rivera J, Herceg Z, Romieu I. Dietary supplementation with polyunsaturated fatty acid during pregnancy modulates DNA methylation at IGF2/H19 imprinted genes and growth of infants. Physiol Genomics 2014;46(23):851-857.

Supplemental Material

Figure S3.4.1. Flow chart of the participant

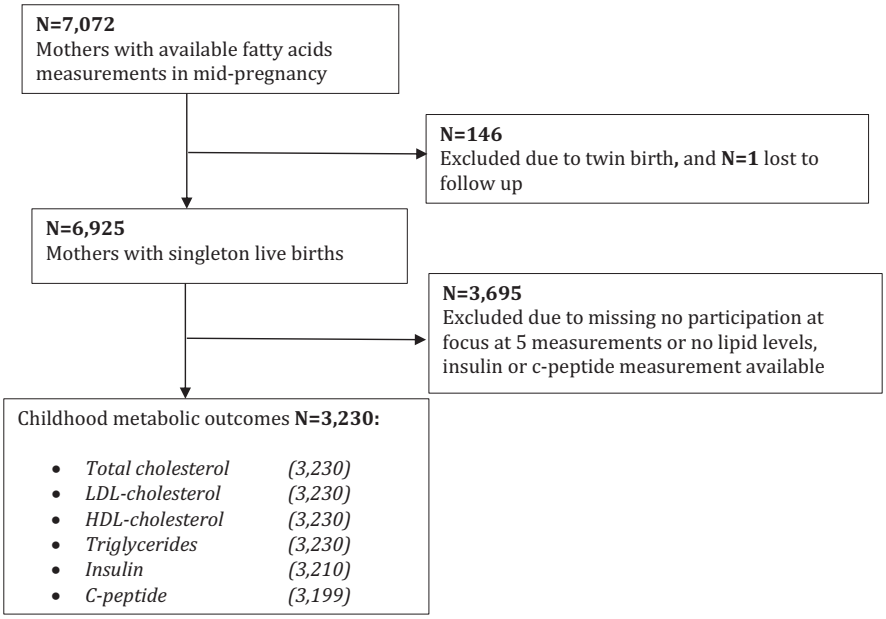


Table S3.4.1. Comparison of subject characteristics between those included and not included in the analyses (N=3,230)[a]

Characteristics	Participation in follow-up N = 3,230	No participation in follow-up N = 3,695	*P*-value
Maternal Characteristics			
Age, (y), median (95% range)	31.0 (20.1, 39.4)	29.6 (18.9, 38.9)	0.1
Gestational age at fatty acid measures, (weeks), median (95% range)	20.5 (17.1, 24.9)	20.5 (18.5, 23.6)	0.04
Pre-pregnancy body mass index, (kg/m2), mean (SD)	23.6 (4.2)	23.7 (4.5)	0.69
Weight gain, (kg), mean (SD)	10.4 (4.9)	10.3 (5.2)	<0.01
Education, higher education No. (%)	1455 (47.7)	1247 (37.5)	<0.01
Ethnicity, European No. (%)	1963 (61.9)	1851 (53.8)	0.04
Parity, nulliparous No. (%)	1808 (56.3)	2036 (55.8)	<0.01
Smoking during pregnancy, No. yes (%)	736 (25.6)	950 (29.4)	0.30
Folic acid supplement use, No. yes (%)	1867 (75.7)	1838 (67.8)	<0.01
Gestational diabetes, No. (%)	27 (0.9)	43 (1.2)	0.11
Gestational hypertensive disorders, No. (%)	176 (5.6)	210 (5.9)	0.17
Birth and infant characteristics			
Males, No. (%)	1650 (51.1)	1850 (50.1)	0.51
Gestational age at birth (weeks), median (95% range)	40.1 (35.8, 42.3)	40.1 (35.0, 42.4)	<0.01
Breastfeeding duration (months), mean (SD)	4.5 (3.9)	4.1 (3.8)	<0.01
Introduction of solid foods No. (%) >6 months	208 (10.1)	185 (10.8)	0.84
N-3 PUFA intake (g/d)	0.6 (0.4)	0.6 (0.4)	0.5
N-6 PUFA intake (g/d)	4.7 (3.0)	4.9 (3.3)	0.31
Childhood outcomes at 6 years			
Body mass index at 6 years (kg/m^2), mean (SD)	16.2 (1.8)	16.2 (1.9)	0.45
TV watching time, No. (%)			
< 2 hours/day	2059 (80.6)	1326 (81.6)	0.50
≥ 2 hours/day	496 (19.4)	299 (18.4)	

[a]Values represent means ± SDs, median (95% range) or number of subjects (valid%).

Table S3.4.2. Comparison of maternal PUFA levels in plasma between those included and not included in the analyses (N=3,320)[a]

Maternal PUFAs levels	Participation in follow-up N = 4,830	No participation in follow-up N = 2,094	P-value
Total PUFA levels			
Absolute levels (mg/L)	708.3 ± 98.8	702.0 ± 100.7	<0.01
Percentage by weight of total sum of fatty acids (%)	43.1 ± 2.0	43.3 ± 2.1	<0.01
Total n-3 PUFA			
Absolute levels (mg/L)	105.4 ± 27.7	101.7 ± 26.9	<0.01
Percentage by weight of total sum of fatty acids (%)	6.5 ± 1.5	6.3 ±1.4	0.6
ALA			
Absolute levels (mg/L)	5.1 ± 1.8	4.9 ± 1.9	<0.01
Percentage by weight of total sum of fatty acids (%)	0.3 ± 0.1	0.3 ± 0.1	0.1
EPA			
Absolute levels (mg/L)	8.8 ± 5.5	8.1 ± 5.2	<0.01
Percentage by weight of total sum of fatty acids (%)	0.5 ± 0.3	0.5 ± 0.3	0.5
DPA			
Absolute levels (mg/L)	12.1 ± 4.4	11.8 ± 4.3	<0.01
Percentage by weight of total sum of fatty acids (%)	0.7 ± 0.2	0.7 ± 0.2	0.8
DHA			
Absolute levels (mg/L)	77.9 ± 20.5	75.3 ± 20.0	<0.01
Percentage by weight of total sum of fatty acids (%)	4.8 ± 1.1	4.7 ± 1.1	0.4
Total n-6 PUFA			
Absolute levels (mg/L)	602.8 ± 89.0	604.3 ± 89.5	<0.01
Percentage by weight of total sum of fatty acids (%)	37.2 ± 2.5	37.6 ± 2.5	<0.01
LA			
Absolute levels (mg/L)	361.1 ± 63.5	361.8 ± 62.3	<0.01
Percentage by weight of total sum of fatty acids (%)	22.3 ± 2.8	22.5 ± 2.8	0.4
GLA			
Absolute levels (mg/L)	1.5 ± 0.7	1.5 ± 0.7	<0.01
Percentage by weight of total sum of fatty acids (%)	0.1 ± 0.1	0.1 ± 0.0	<0.01
EDA			
Absolute levels (mg/L)	8.5 ± 1.9	8.5 ± 1.9	<0.01
Percentage by weight of total sum of fatty acids (%)	0.5 ± 0.1	0.5 ± 0.1	<0.01
DGLA			
Absolute levels (mg/L)	61.0 ± 16.5	60.1 ± 17.1	<0.01
Percentage by weight of total sum of fatty acids (%)	3.7 ± 0.7	3.7 ± 0.8	0.1
AA			
Absolute levels (mg/L)	156.4 ± 32.6	157.2 ± 32.9	<0.01
Percentage by weight of total sum of fatty acids (%)	9.6 ± 1.6	9.8 ± 1.6	0.1
DTA			
Absolute levels (mg/L)	6.9 ± 2.2	7.1 ± 2.2	<0.01
Percentage by weight of total sum of fatty acids (%)	0.4 ± 1.1	0.4 ± 0.1	0.1
Total n-6/n-3 PUFAs ratio			
Absolute levels (mg/L)	6.1 ± 1.7	6.3 ± 1.7	0.2
Percentage by weight of total sum of fatty acids (%)	-	-	

[a]Values represent mean ± SD.

Abbreviations: ALA: α-linolenic acid; AA: arachidonic acid; DGLA dihomo-gamma-linolenic acid; DHA: docosahexaenoic acid; DGLA dihomo-γ-linolenic acid; DPA: docosapentaenoic acid; DTA: docosatetraenoic acid; EDA: eicosadienoic acid; EPA: eicosapentaenoic acid; GLA: γ-linolenic acid; LA: linoleic acid; PUFA; polyunsaturated fatty acid.

Table S3.4.3. Correlation between maternal PUFAs (N=3,230)[a]

Maternal PUFA	Total PUFA	Total n-3 PUFA	ALA	EPA	DPA	DHA	Total n-6 PUFA	LA	GLA	EDA	DGLA	ARA	DTA
Total PUFA	1												
Total n-3 PUFA	0.47*	1											
ALA	0.38*	0.33*	1										
EPA	0.22*	0.77*	0.27*	1									
DPA	0.38*	0.65*	0.35*	0.48*	1								
DHA	0.46*	0.97*	0.21*	0.65*	0.51*	1							
Total n-6 PUFA	0.96*	0.22*	0.31*	0.00	0.21*	0.21*	1						
LA	0.81*	0.08*	0.35*	-0.07*	0.07*	0.07*	0.87*	1					
GLA	0.35*	0.16*	0.20*	0.12*	0.27*	0.10*	0.34*	0.10*	1				
EDA	0.58*	0.11*	0.28*	-0.06*	0.13*	0.11*	0.61*	0.62*	0.14*	1			
DGLA	0.60*	0.22*	0.30*	0.10*	0.32*	0.16*	0.59*	0.32*	0.51*	0.50*	1		
AA	0.65*	0.32*	0.00	0.11*	0.23*	0.35*	0.62*	0.21*	0.36*	0.11*	0.36*	1	
DTA	0.45*	0.03	0.08*	-0.11*	0.32*	-0.01	0.49*	0.16*	0.51*	0.27*	0.55*	0.57*	1

[a]Values are Pearson correlation coefficients. *P- value <0.06.

Abbreviations: ALA: α-linolenic acid; AA: arachidonic acid; DGLA dihomo-γ-linolenic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; DTA: docosatetraenoic acid; EDA: eicosadienoic acid; EPA: eicosapentaenoic acid; GLA: γ-linolenic acid; LA: linoleic acid; PUFA; polyunsaturated fatty acid.

Table S3.4.4. Maternal plasma n-3 PUFA levels and childhood c-peptide levels (N=3,230)[a]

Maternal n-3 PUFA in SDS	Differences in childhood c-peptide levels (95% confidence interval) in SDS
Total n-3 PUFA	
Adjusted model[2]	0.04 (0, 0.08)*
Childhood body mass index[3]	0.04 (0.01, 0.08)*
ALA	
Adjusted model	-0.01 (-0.04, 0.03)
Childhood body mass index	-0.01 (-0.04, 0.04)
EPA	
Adjusted model	0.02 (-0.02, 0.06)
Childhood body mass index	0.02 (-0.02, 0.06)
DPA	
Adjusted model	0.03 (-0.01, 0.07)
Childhood body mass index	0.03 (-0.01, 0.07)
DHA	
Adjusted model	0.04 (-0.01, 0.07)
Childhood body mass index	0.04 (0, 0.07)*

[a]Values are regression coefficients (95% CIs) that reflect the difference in SDS of childhood c-peptide levels per SD change in maternal n-3 PUFA levels, respectively.

[b]Adjusted model is adjusted for maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, smoking habits during pregnancy, folic acid supplement use and total caloric intake during pregnancy and pregnancy complications, and child's sex, gestational age-adjusted birth weight standard-deviation scores, breastfeeding duration, timing of introduction of solid foods, and TV watching time. [c]Childhood BMI model is additionally adjusted for body mass index standard-deviation scores at 6 years. *P-value<0.05.

Abbreviations: ALA: α-linolenic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; PUFA; polyunsaturated fatty acid.

Table S3.4.5. Maternal plasma n-6 PUFA levels and childhood c-peptide levels (N=3,230)[a]

Maternal n-6 PUFA in SDS	**Differences in childhood c-peptide levels (95% confidence interval) in SDS**
Total n-6 PUFA	
Adjusted model[2]	-0.02 (-0.06, 0.02)
Childhood BMI model[3]	-0.02 (-0.06, 0.02)
LA	
Adjusted model	-0.01 (-0.05, 0.03)
Childhood BMI model	-0.01 (-0.05, 0.03)
GLA	
Adjusted model	0.01 (-0.02, 0.05)
Childhood BMI model	0.01 (-0.02, 0.05)
EDA	
Adjusted model	-0.01 (-0.05, 0.02)
Childhood BMI model	-0.01 (-0.05, 0.02)
DGLA	
Adjusted model	-0.01 (-0.05, 0.02)
Childhood BMI model	-0.01 (-0.05, 0.02)
AA	
Adjusted model	0 (-0.04, 0.04)
Childhood BMI model	-0.01 (-0.04, 0.03)
DTA	
Adjusted model	-0.02 (-0.05, 0.02)
Childhood BMI model	-0.02 (-0.05, 0.02)

[a]Values are regression coefficients (95% CIs) that reflect the difference in SDS of childhood c-peptide levels per SD change in maternal n-6 PUFA levels, respectively. [b]Adjusted model is adjusted for maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, smoking habits during pregnancy, folic acid supplement use and total caloric intake during pregnancy and pregnancy complications, and child's sex, gestational age-adjusted birth weight standard-deviation scores, breastfeeding duration, timing of introduction of solid foods, and TV watching time. [c]Childhood BMI model is additionally adjusted for body mass index standard-deviation scores at 6 years. *P-value<0.05. Abbreviations: AA: arachidonic acid; DGLA dihomo-gamma-linolenic acid; DGLA dihomo-γ-linolenic acid; DTA: docosatetraenoic acid; EDA: eicosadienoic acid; GLA: γ-linolenic acid; LA: linoleic acid; PUFA; polyunsaturated fatty acid.

Chapter 4

General discussion

Introduction

Cardio-metabolic disease is the leading cause of death worldwide.[1] The developmental origins hypothesis proposes that cardio-metabolic disease at least partly originates during early life.[2] This hypothesis suggests that adverse factors during pregnancy may adversely affect the fetal development, and thereby increase the risk of developing diseases later in life.

Adverse exposures in pregnancy such as poor maternal nutrition might influence the composition and distribution of fetal tissues and lead to alterations in cardio-metabolic structure and function.[3] The Dutch famine study showed that severe fetal undernutrition in different periods of pregnancy may lead to an increased risk of obesity and coronary heart disease in later life.[4] However, less is known about the influence of maternal malnutrition on offspring outcomes. Maternal plasma fatty acids are important for fetal growth and development.[5] Fatty acids are natural components of fats and oils and are derived from triglycerides or phospholipids. Based on their chemical structure they can be differentiated into groups: saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). The fetus can synthesize some SFAs and MUFAs de novo, but PUFAs must be provided by placental transfer because humans cannot synthesize them. Therefore, the placental transfer of PUFAs is a key issue in mobilizing fatty acids for fetal growth and development.[6] Since PUFAs are essential nutrients, dietary fatty acids intake during pregnancy must be adequate to meet the demands of both the mother and the developing fetus. A suboptimal maternal fatty acids profile during pregnancy is associated with adverse maternal and fetal pregnancy outcomes.[7,8] However, less is known about the influence of maternal fatty acids status during pregnancy on long term offspring cardio-metabolic health.

The aim of the studies presented in this thesis was to identify the influence of maternal fatty acid levels measured in mid-pregnancy on cardio-metabolic health outcomes in mothers and their children. This chapter provides a general discussion of the main findings of the studies presented in this thesis discusses general methodological issues and provides suggestions for future research.

Interpretation of main findings

Maternal environment

Prepregnancy body mass index, gestational weight gain and fatty acid

Maternal prepregnancy obesity is a major public health problem worldwide, and is associated with adverse maternal and fetal pregnancy outcomes.[9] In addition, excessive gestational weight gain has been linked to complications during pregnancy, including diabetes mellitus, gestational hypertensive disorders and delivering large size for gestational age infants.[10] Both maternal prepregnancy obesity and excessive gestational weight gain are associated with an increased risk of childhood obesity and related adverse cardio-metabolic profile.[11]

A growing body of evidence indicates that increased body weight during pregnancy is associated with altered fatty acid status. Obesity during pregnancy may lead to higher circulating free fatty acid levels caused by dyslipidaemia and insulin resistance.[12] Results from previous studies among adults have shown that a higher body mass index is associated with higher concentrations of SFA, lower levels of n-3 PUFA and higher n-6/n-3 PUFAs ratio.[13,14] A previous study suggested that women who were obese before pregnancy had higher SFA concentrations and lower n-3 PUFA concentrations in pregnancy.[15] Not much is known about the associations between prepregnancy body mass index, gestational weight gain and specific groups of fatty acids.

We examined whether prepregnancy body mass index and gestational weight gain are associated with plasma SFA, MUFA, n-3 and n-6 PUFA concentrations in mid-pregnancy. We showed positive associations between prepregnancy body mass index and total SFA, whereas positive associations were observed only between prepregnancy body mass index and palmitoleic acid concentrations These results are in line with those observed in non-pregnant adults. Among individual n-3 PUFAs, prepregnancy body mass index was associated with lower α-linolenic acid and eicosapentaenoic acid concentrations but not with total n-3 PUFAs. Furthermore, obese women showed higher total n-6 PUFA and especially higher dihomo-γ-linolenic acid and arachidonic acid concentrations In women with excessive gestational weight gain, independent of their prepregnancy body mass index, concentrations of higher total SFA and MUFA were found. Among n-3 PUFAs, positive associations were observed between gestational weight gain with α-linolenic acid and eicosapentaenoic acid concentrations whereas among n-6 PUFAs positive associations were observed between gestational weight gain and total n-6 PUFAs and dihomo-γ-linolenic acid concentrations Both obese women and women with excessive weight gain had a higher n-6/n-3 PUFA

ratio. Thus, our findings suggest that obesity and excessive weight gain during pregnancy are associated with an adverse fatty acids profile (**Table 4.1**).

The mechanisms underlying the association between prepregnancy body mass index, gestational weight gain and adverse fatty acid concentrations are largely unknown. Several mechanisms may be involved. Increased prepregnancy weight may alter the absorption and metabolism of circulating fatty acid levels.[15] Also, the diet composition of overweight and obese women during pregnancy may differ comparted to normal weight women with larger amounts of food high in total fat and SFAs. However, higher fatty acids may also result in changes in neuroendocrine functioning and energy metabolism leading to increased risk of obesity throughout pregnancy.[16] We were not able to study the direction and causality of the observed associations, which therefore remains to be further explored in future studies with repeated maternal weight gain and fatty acids concentrations available throughout pregnancy.

Table 4.1. Body mass index, gestational weight gain and fatty acids

Exposure	Fatty acid levels during pregnancy			
Prepregnancy body mass index	↑SFA		↓n-3 PUFA	↑n-6 PUFA
Gestational weight gain	↑SFA	↑MUFAs		↑n-6 PUFA

Maternal vascular adaptations

Pregnancy is characterized by major cardiovascular adaptations, including marked decreases in systemic vascular resistance and an increase in maternal cardiac output and total blood volume.[17] Hypertensive disorders in pregnancy, including preeclampsia and gestational hypertension are associated with increased risk of maternal and perinatal morbidity and mortality.[18] A numbers of studies have examined whether maternal diet during pregnancy might influence risks of gestational hypertensive disorders. Among adults, it has been suggested that an adverse fatty acids profile, characterized by especially higher SFAs and lower PUFAs, is associated with cardiovascular disease.[19,20] Results from observational and randomized controlled studies suggest that higher maternal levels of n-3 PUFA during pregnancy are associated with a lower risk of preeclampsia, whereas higher levels of SFA and n-6 PUFA are associated with an increased risk of preeclampsia.[21,22] Not much is known about the effect of maternal fatty acids status on blood pressure development and placental function throughout pregnancy. Alterations in maternal hemodynamic adaptations during pregnancy may partly underlie the observed associations with gestational hypertensive disorders.

We examined whether plasma SFA, MUFA, n-3 and n-6 PUFA concentrations during pregnancy affect maternal blood pressure development, umbilical and uterine artery resistance indices and the risk of gestational hypertensive disorders. Results presented in this thesis showed that higher SFAs, but not MUFAs, were associated with a higher blood pressure from first trimester onwards. Higher total maternal n-3 and n-6 PUFAs and especially docosahexaenoic acid and linoleic acid concentrations were associated with a lower blood pressure throughout pregnancy. Fatty acid concentrations were not consistently associated with second and third trimester placental vascular resistance index. Only higher maternal SFA concentrations were associated with the risk of gestational hypertensive disorders. These findings suggest that an adverse maternal fatty acids profile is associated with higher maternal blood pressure levels throughout pregnancy and might also increase the risk of gestational hypertensive disorders (**Table 4.2**).

Table 4.2. Fatty acids and hemodynamic adaptations during pregnancy

Exposure	Maternal hemodynamic adaptations	
SFA	↑ Blood pressure	↑ Gestational hypertensive disorders
n-3 PUFA	↓ Blood pressure	
n-6 PUFA	↓ Blood pressure	

The mechanism by which fatty acids may influence gestational hemodynamic adaptations are not well understood. First, it has been suggested that higher SFAs are associated with a higher blood pressure by impairing endothelial function with higher levels of cholesterol which accumulate in the arterial walls and with increased sympathetic nervous system activities. Hypotensive effect of n-3 PUFAs could be related to increased production of vasodilators such as prostaglandin I_3 and reduction of vasoconstrictors such as thromboxane A_2.[23,24] Arachidonic acid, n-6 PUFAs, has opposite effects of n-3 PUFAs on eicosanoid biosynthesis, which in turn increase blood pressure.[25] Second, adverse fatty acid levels might contribute to placental dysfunction and might damage uteroplacental tissues by increasing production and secretion of several proinflammatory cytokines that might cause endothelial dysfunction and induce oxidative stress.[26,27] Since PUFAs, including docosahexaenoic, eicosapentaenoic, linoleic and arachidonic acids, are natural ligands for peroxisome proliferator-activated receptors, they are known to be important for placental development and vascularization, and they might improve uterine artery blood flow.[28] In this study we did not observe association with resistance indices; however these associations may be present in higher risk populations. Third, since n-3 PUFAs are precursors of pros-

taglandins and thromboxanes, they may contribute to endothelial cell dysfunction seen in gestational hypertensive disorders because of their role during pregnancy to maintain the fluidity, permeability and conformation of membranes.[29] In contrary, proinflammatory effects of arachidonic acid, n-6 PUFAs, promote vasoconstriction and consequently lead to preeclampsia.[25] Finally, the observed associations may be explained by socio-demographic and lifestyle related characteristics. In our study, we adjusted for multiple confounders. However, residual confounding might still be an issue. Future studies are needed to obtain further insight into these associations, the underlying mechanism and causality.

Childhood outcomes

Fetal life is an important period for the development of cardio-metabolic diseases later in life. PUFAs, particularly n-3 and n-6 PUFAs are critical nutrients during pregnancy and might be involved in fetal development and childhood cardio-metabolic risks.[5]

Childhood adiposity

The prevalence of childhood obesity has increased dramatically over the past several decades.[30] Obesity in childhood is associated with established risk factors for cardio-metabolic diseases in later life, including elevated blood pressure, dyslipidemia, metabolic syndrome, and diabetes mellitus.[31] There is accumulating evidence that fetal programming by the nutritional environment in utero influences body composition later in life. Elements of maternal diet during pregnancy are commonly regarded as a major factor contributing to childhood obesity. PUFAs, in particular n-3 and n-6 PUFAs, play an important role in fetal development and previous studies suggested that a maternal diet rich in PUFAs might influence the determination of body composition in the offspring.[32,33] It was shown that n-3 PUFAs, particularly eicosapentaenoic and docosahexaenoic acids, inhibit adipose tissue development, while n-6 PUFAs, counteract this process. In addition, positive associations were found between n-6/n-3 PUFAs ratio and body mass index during the first 10 years of life.[34] Thus far, studies focused on the associations of maternal PUFAs profile with body mass index in children show inconsistent results and suffer from having a small sample size.[35,36] Also, previous studies mainly focused on childhood body mass index, which does not distinguish lean mass from fat mass.[37]

We examined whether specific maternal plasma n-3 PUFA and n-6 PUFA concentrations and the ratio of n-6/n-3 PUFA, during the second trimester of pregnancy are associated with childhood body mass index and specific body fat measures, including fat mass percentage and android/gynoid fat ratio measured by dual-energy x-ray ab-

sorptiometry (DXA) and pre-peritoneal abdominal fat area measured by ultrasound, which is a measure of visceral fat mass. We showed that higher maternal total n-3 PUFA concentrations and especially eicosapentaenoic, docosapentaenoic and docosahexaenoic acids, were associated with lower childhood total body fat percentage and lower android/gynoid fat mass ratio, but not with childhood body mass index and abdominal pre-peritoneal fat mass area. Similarly, results from a US pregnancy cohort among 1,250 mother-child pair showed that higher docosahexaenoic and eicosapentaenoic acid concentrations were associated with a lower subcutaneous fat mass at the age of 3 years.[32] Among n-6 PUFA concentrations we observed that higher maternal total and especially dihomo-γ-linolenic acid levels were associated with a higher childhood total body fat percentage, android/gynoid fat mass ratio and abdominal pre-peritoneal fat mass area, but not with childhood body mass index. These associations were only partly explained by maternal and childhood sociodemographic and lifestyle-related characteristics. Our finding suggests that lower maternal n-3 PUFA concentrations and higher n-6 PUFA concentrations during pregnancy are associated with higher total body fat and abdominal fat levels in childhood (**Table 4.3**).

Table 4.3. Maternal PUFA and childhood adiposity

Exposure	Childhood adiposity			
Maternal n-3 PUFAs	↓BMI	↓TFM	↓AvsG	
Maternal n-6 PUFAs		↑TFM	↑AvsG	↑PPAREA
Maternal n-6/n-3 PUFAs ratio		↑TFM	↑AvsG	↑PPAREA

Childhood blood pressure

The prevalence of hypertension among children is increasing in accordance with rising obesity rates.[38,39] High blood pressure in children is a risk factor for developing cardio-metabolic disease later in life. Animal studies found that maternal n-3 PUFAs deficiency during pregnancy is associated with higher blood pressure in the offspring later in life.[40] Not much is known about the associations of different maternal n-3 and n-6 PUFAs during pregnancy with childhood blood pressure levels.

We examined whether maternal specific n-3 and n-6 PUFA concentrations and n-6/n-3 PUFA ratio during pregnancy are associated with childhood systolic and diastolic blood pressure, measurements taken in the offspring at 6 years of age. Results presented in this thesis suggests that higher total maternal n-3 PUFA and especially docosahexaenoic acid concentrations were associated with lower childhood systolic blood pressure, but not with childhood diastolic blood pressure. Among n-6 PUFAs, we observed that total maternal n-6 PUFA wt% but not individual fatty acids, were

positively associated with childhood systolic blood pressure, but not with childhood diastolic blood pressure. Also, we observed that a higher n-6/n-3 PUFA ratio was associated with higher childhood systolic blood pressure. These associations were only partly explained by pregnancy and childhood characteristics. Our finding suggests for the first time that higher maternal plasma n-3 PUFA and lower n-6 PUFA concentrations during mid-pregnancy are associated with a lower systolic blood pressure in childhood. Thus, increased blood pressure later in life may be associated with altered maternal concentrations during pregnancy of n-3 and n-6 PUFAs, or unbalanced n-6/n-3 PUFAs ratio (**Table 4.4**).

Table 4.4. Maternal PUFA and childhood blood pressure

Exposure	Childhood blood pressure
Maternal n-3 PUFA	↓ Systolic blood pressure
Maternal n-6 PUFA	↑ Systolic blood pressure
Maternal n-6/n-3 PUFA ratio	↑ Systolic blood pressure

Childhood lipids and insulin levels

A suboptimal lipids profile and an impaired insulin and glucose metabolism early in life have been recognized as important risk factors for cardio-metabolic disease later in life.[41] Animal studies have shown that a maternal diet containing higher levels of PUFA during pregnancy improves the offspring's lipid profile and insulin metabolism in the postnatal period.[42-46] However, few studies among human populations have assessed possible effects of maternal specific n-3 and n-6 PUFAs during pregnancy on long-term offspring lipid and insulin metabolism.

Therefore, we examined whether maternal plasma n-3 and n-6 PUFA levels during pregnancy are associated with childhood lipids and insulin levels. In this thesis we observed that higher maternal total n-3 PUFA levels and especially docosahexaenoic acid levels were associated with higher childhood total-cholesterol, HDL-cholesterol and insulin levels. In addition, higher maternal eicosapentaenoic acid levels were associated with a higher childhood HDL-cholesterol, whereas higher docosapentaenoic acid levels were associated with higher childhood insulin levels. Maternal total n-6 PUFA levels were not associated with childhood outcomes. Higher levels of the individual n-6 PUFAs, eicosadienoic and dihomo-γ-linolenic acids were associated with a lower childhood HDL-cholesterol, whereas higher arachidonic acid levels were associated with higher childhood total-cholesterol and HDL-cholesterol levels. Partly in line with our findings, a small study among 242 pregnant women showed that higher levels of maternal docosapentaenoic acid during pregnancy were associated with higher total-cholesterol and LDL-cholesterol levels in the offspring.[46] Higher mater-

nal arachidonic acid levels were associated with lower childhood triglycerides and LDL-cholesterol levels, but no associations were observed for other n-6 PUFA levels. Another small study performed among 259 children suggests that higher availability of γ-linolenic and dihomo-γ-linolenic acids at birth were associated with reduced levels of plasma triacylglycerol and insulin resistance.[47] The observed associations in this thesis were not explained by maternal and childhood socio-demographic and lifestyle related characteristics or childhood body mass index. Our finding suggests that higher maternal total n-3 PUFAs and specifically docosapentaenoic acid levels during pregnancy are associated with higher childhood total-cholesterol, HDL-cholesterol and insulin levels. Among maternal n-6 PUFAs only individual, not total maternal n-6 PUFA levels, tended to be associated with childhood lipids and insulin levels. Thus, these results suggest that specific maternal n-3 and n-6 PUFA levels during pregnancy could be important for long-term offspring lipid and insulin/glucose metabolism (**Table 4.5**).

Table 4.5. Maternal PUFA and childhood lipids and insulin levels

Exposure	**Childhood lipids and insulin levels**			
Maternal n-3 PUFAs	↑ Total cholesterol	↑ HDL-cholesterol		↑ Insulin
Maternal n-6/n-3 PUFA ratio		↓ HDL-cholesterol	↑ Triglycerides	↓ Insulin

Underlying mechanisms

During pregnancy, an adequate maternal n-3 and n-6 PUFAs profile is essential in maternal diet as it affects different components of fetal development, including structures of the cell membranes, organs function and metabolism.[48] Among n-3 and n-6 PUFAs, a-linolenic acid and linoleic acid are the parent fatty acids and through elongation and desaturation of these fatty acids in the human body, a series of longer-chain fatty acids can be synthesized, such as docosahexaenoic acid and arachidonic acid.[48] In this thesis we therefore specifically studied these total and individual maternal n-3 PUFAs and n-6 PUFAs. Several potential mechanisms have been proposed in the relation between maternal total and individual n-3 and n-6 PUFAs during pregnancy and childhood cardio-metabolic outcomes.

Both maternal levels of n-3 and n-6 PUFAs affect the proliferation and differentiation of fetal adipose cells through their role as precursors of eicosanoids such as prostaglandins, thromboxanes and leukotrienes.[49] N-3 PUFA inhibits cyclic adenosine monophosphate production through prostaglandins, leading to inhibition of fetal adipocytes differentiation.[50] On the contrary, eicosanoids derived from n-6 PUFAs, appears to be highly adipogenic promoting the differentiation of fetal preadipocytes into mature and functional adipocytes.[49] We observed that higher maternal n-3 PUFAs

during pregnancy, which inhibit differentiation of adipocyte cells, were associated with lower childhood fat mass. Also, we observed that especially dihomo-γ-linolenic acid levels, which stimulate the proliferation of fetal adipocytes, were associated with higher childhood fat mass.

We observed that higher maternal plasma n-3 PUFA and lower n-6 PUFA levels during pregnancy were associated with a lower systolic blood pressure in childhood. Several mechanisms have been proposed which may partly explain these observed associations. The hypotensive effect of maternal n-3 PUFAs may be related to improved fetal endothelial vasodilator function and reduced pressor reactivity of resistance vessels.[51] It has been suggested that docosahexaenoic acid enhanced dilatory responses by attenuating constrictor responses to noradrenaline, in relation with a decrease in plasma norepinephrine which mechanism might be responsible for a reduction in blood pressure.[52] In addition, they have hypotensive properties through stimulation of prostaglandins that control sodium and water excretion, inhibition of the vasoconstrictor thromboxane and decrease of the response to vasopressor hormones.[53-55] Also, n-3 PUFAs during fetal development may stimulate adenosine triphosphate release from the endothelium and increase vasodilation by stimulating nitric oxide release.[56] On the contrary, n-6 PUFAs during fetal development may act as precursors of the second series of prostaglandins and thromboxanes, which can stimulate offspring vasoconstriction and increase offspring blood pressure.[57]

We observed that higher maternal total n-3 PUFAs and specifically DHA levels during pregnancy are associated with higher childhood total-cholesterol, HDL-cholesterol and insulin levels. Only individual maternal n-6 PUFAs, not total maternal n-6 PUFA levels, tended to be associated with childhood lipids and insulin levels. The effect of fatty acids profile during fetus development on childhood metabolic outcomes might be through epigenetic mechanisms or structural adaptations that lead to changes in biological mechanisms of liver and adipose tissue which are strongly associated with development of dyslipidemia, insulin resistance, and cardiovascular diseases. Some other mechanisms have also been proposed. The lipid lowering action of n-3 PUFAs seems to occur through a reduction of hepatic synthesis of triacylglycerol and very-low-density-lipoprotein.[58] As a part of the cell membrane, n-3 PUFAs during fetal development may be able to regulate insulin secretion from pancreatic b-cells directly by altering lipid raft structure.[59] N-6 PUFAs might impair the lipid metabolism and change the fatty liver content by increasing blood levels of total cholesterol and triacylglycerols.

Rather than the absolute amount of n-3 PUFA and n-6 PUFAs during pregnancy, especially the ratio of n-3/n-6 PUFAs may be of importance. We observed that a higher maternal n-6/n-3 ratio was associated with an adverse childhood cardio-metabolic

profile. Increased intake of n-6 PUFAs might inhibit n-3 PUFAs incorporation into membranes and reduce the production of anti-inflammatory mediators, since the enzymes involved in their metabolism are the same. Based on our findings, it seems that increasing maternal n-3 PUFAs, with a reduced n-6/n-3 PUFAs ratio may be beneficial for cardio-metabolic outcomes in the offspring. However, because of the observational design, residual confounding due to unmeasured maternal and childhood socio-demographic and lifestyle related determinants might still be an issue. Thus, further research using sophisticated study designs, including randomized controlled trials, are needed to obtain further insight into the causality of these observed associations and the potential to influence long term health outcomes of offspring.

Methodological considerations

Specific methodological considerations of the studies presented in this thesis have been described in **Chapter 2** and **Chapter 3** of this thesis. In the following paragraphs, general methodological considerations regarding selection bias, information bias and confounding are discussed.

Selection bias

Selection bias may occur if the association between the determinant and outcome of interest is different in subjects who participate in the study and those who were eligible but do not participate in the study. In total, 61% of all eligible children in the study area participated at birth in the Generation R Study. This non-response due to non-participation and missing values at baseline among the participants is not likely to be at random. The percentages of women from ethnic minorities and lower socio-economic status were lower than expected from the population figures in Rotterdam.[60] Also, the percentages of women with gestational hypertension and preeclampsia, or children born preterm or with a low birth weight were lower, which might indicate a selection towards a healthy population. This selection towards a more affluent and healthy population may have led to lower prevalence rates, and subsequently reduced statistical power. Also, the selection mechanisms at baseline may affect the generalizability of our results due to differences in frequency rates in exposures and outcomes. However, several studies have shown that effect estimates in association studies are not markedly affected by selective non-participation at baseline in large cohort studies.[61,62]

Selection bias in our studies might also occur due to selective loss to follow-up if the associations were different between those included and not included in the

analyses, but this seems unlikely since loss to follow-up at birth in the presented studies was low. At the age of 6 years, children and their mothers were invited to participate in detailed body fat and cardio-metabolic follow-up measurements. The response rate at this follow-up was approximately 70%. A lower percentage of children participated in blood sample measurements at the age of 6 years, which was mainly due to non-consent for venous puncture or crying of the child. Mothers from children who did not visit the research center more frequently had unhealthy lifestyle habits, lower total n-3 PUFA levels and higher n-6/n-3 PUFAs ratio and were less well educated than the total study population. Overall, the selective loss to follow-up towards a more healthy population may have affected the effect estimates presented in this thesis, but this bias is difficult to quantify.

Information bias

Information bias, otherwise known as misclassification, is a bias in which error occurs in the measurement of an exposure or outcome.[63] Misclassification refers to measurement error of either determinant or outcome and might be classified into two types: differential or non-differential. Differential misclassification occurs when the error rate or probability of being misclassified differs across groups of study subjects. In differential misclassification the determinant and outcome status are related and this type of measurement error may lead to either overestimated or underestimated effect estimates.[63] In our study, exposure data were collected longitudinally and before assessment of the outcomes. Also, both mothers and their children were not aware of the specific research questions addressed in this thesis. This makes differential misclassification of the exposure unlikely.

Non-differential misclassification can occur in a number of ways, and therefore non-differential misclassification might have occurred. In non-differential misclassification the determinant status is not related to the outcome status, and vice versa. Self-reporting is a common approach for gathering data in epidemiologic and medical research. Non-differential misclassification generally leads to an underestimation or dilution of the effect estimates. For example, in the studies presented in this thesis, information of maternal prepregnancy weight and maximum weight during pregnancy was self-reported. Self-reported weight tends to be underestimated especially in case of higher maternal weight, which might have led to an underestimation of observed effects for maternal prepregnancy body mass index and maximum gestational weight gain. In most of the studies presented in this thesis, the outcome was assessed using medical records, well-trained research nurses or standardized hands-on assessments of body composition and cardio-metabolic development.

Furthermore, the observers were not aware of the exposure status, which makes differential misclassification of the outcomes less likely.

Confounding

A confounding variable is an extraneous variable that correlates with both the dependent variable and the independent variable, and this variable is not an intermediate variable in the causal pathway between the exposure and the outcome.[63] The observational design of the study, such as the Generation R Study, also increases the risk of confounding. If a confounding factor is not taken into account, this may lead to a biased effect estimate of the association between the determinant and the outcome. To control for confounding in this thesis, we had information available on many potential confounders. We selected potential confounders based on previous literature, their associations with the outcomes of interest or a change in effect estimate of more than 10%. Our main interest was the effect of maternal fatty acid levels on childhood cardio-metabolic outcomes, for which we had information on multiple maternal and childhood socio-demographic and lifestyle related characteristics. Maternal fatty acids status might reflect other contributing dietary components and the results could be confounded by similarities between maternal diet in pregnancy and the child's postnatal dietary exposures. In a subgroup of our study we performed an additional analysis to explore whether the associations were explained by infant PUFAs intake at 13 months and we observed that additional adjustment for PUFA intake in infants did not materially affect the observed associations. Nevertheless, as in all observational studies, results could still be affected through residual confounding by unmeasured confounders or imperfect measurement of measured confounders. Residual confounding may have led to an overestimation of the observed effect estimates. In most of the studies presented in this thesis, adjustment for potential confounders only moderately affected the effect estimates, which suggest that the observed associations are possibly true associations between the determinants and the outcomes.

Future research

Cardio-metabolic diseases are major health problems in developed countries. Previous studies have shown that clinical manifestations of cardio-metabolic diseases occur mainly in adulthood, but the onset of these diseases may occur early in life.[64-66] We described the associations of maternal fatty acid levels during pregnancy with cardio-metabolic outcomes in mothers and their children. These results give

more insights in the effects of maternal fatty acids on early development of cardio-metabolic disease and its risk factors. However, the observed effect estimates for the associations were small, and are mainly of interest from a developmental perspective. However, subclinical differences in risk factors for cardio-metabolic disease in childhood may be related to the development of cardio-metabolic disease in later life. It is important to perform long-term follow-up studies from early life into young adulthood in order to examine whether the associations of maternal fatty acid levels during pregnancy with childhood cardio-metabolic disease persist in later life.

More detailed assessment of the exposure needs to be included in future studies. Our assessment of fatty acids status was based on a single measure in blood plasma in mid-pregnancy. One measurement, although indicative, is not a reliable reflection of a mother's long-term fatty acids status. Other nutrients that are associated with cardio-metabolic development and that simultaneously affect fatty acids status should also be taken into account when assessing these associations. Therefore, further studies with more than one measurement of fatty acids status during pregnancy and controlling for other nutrients status are needed. It is also not possible to determine whether the developing fetus was exposed to the same levels of PUFA that were measured in the maternal samples. The transfer of PUFAs from the maternal to fetal circulation by the placenta occurs via several mechanisms including passive and facilitated diffusion.

Main maternal outcomes studied in this thesis were maternal blood pressure development, and umbilical and uterine artery resistance indices. The mechanisms by which fatty acids affect blood pressure during pregnancy remain controversial and not much has been studied about the associations of SFAs, MUFAs and PUFAs with placental vascular development. Further research is needed to address the effect of fatty acids during pregnancy with blood pressure and placental resistance index measurements in higher risk populations. Main childhood outcomes were childhood blood pressure, body composition and cardio-metabolic biomarkers. Further additional measurements of childhood body composition and cardio-metabolic development might provide further insight in the underlying mechanism linking maternal fatty acids as early life exposures to obesity and cardio-metabolic diseases in later life. We measured body composition using Dual-energy-X-ray absorptiometry (DXA), a technique that has previously been utilised in only one study investigating the relationships between maternal PUFAs profile and childhood body fat mass. Future studies are needed for replication. In the pathogenesis of atherosclerosis, endothelial dysfunction and impaired vascular reactivity induced by dyslipidemia play an important role. Ultrasound assessment of endothelial function and intima media thickness may be used as preclinical markers of atherosclerosis. The microvasculature is an

important component related to hypertension. Using retinal vascular imaging the microvasculature in children can be studied. Also, imaging techniques, such as magnetic resonance imaging, are of interest to obtain further insight in detailed body fat distribution and cardiovascular development. Thus, imaging techniques, such as coronary circulation magnetic resonance imaging, are of interest to obtain further insight in detailed cardiovascular development.

Due to the observational design of our studies, we cannot establish causality of the observed associations. We had no information available on mothers' *FADS1* or *FADS2* genes, which encode for desaturase enzymes involved in the conversion of PUFAs. In future, Mendelian randomization studies may also help to establish causality for the observed associations. These studies use genetic variances, which are robustly associated with the exposure of interest and are not affected by confounding, to examine whether the exposure is causally related to outcome. Randomized controlled trials are also a preferred study design to establish causality. Previous randomized controlled trials have mainly focused on the associations of n-3 PUFA with childhood adiposity and have found no association.[67,68] The INFAT randomized trial showed that the combination of an increased n-3 PUFA and a reduced arachidonic acid, n-6 PUFA, dietary intake through supplementation during the perinatal period does not affect total subcutaneous fat mass during the first year of life.[36] A randomized controlled trial among 533 pregnant Danish women showed no effects of n-3 PUFA supplementation during the third trimester of pregnancy on insulin levels in 19-year-old offspring.[69] No randomized controlled trials have been conducted focused on fatty acids levels during pregnancy and pregnancy complications. Most of previous clinical trials had small sample sizes and confounding might still have influenced the results since environmental and genetic factors might not be equally distributed between groups. Thus, further high-quality clinical trials are needed to establish whether fatty acid levels during pregnancy affect maternal and childhood outcomes. More evidence is needed to make proper recommendations for fatty acids consumption during prenatal care.

Clinical implications

In this thesis we identified maternal fatty acid levels that might influence maternal and childhood cardio-metabolic outcomes. These findings may be important for identification of high-risk children and for the development of preventive strategies or interventions already from early stages life onwards. Based on our findings, maternal fatty acids status during pregnancy seems to be important for pregnant

women and childhood development. Cardio-metabolic prevention strategies should start as early in life as possible and should focus on maternal nutritional status during pregnancy by improving fatty acids profile. Therefore, clinical practice should consider the importance of diet during pregnancy with emphasizing an adequate consumption of maternal fatty acids.

References

1. Mendis S PP, Norrving B. Global atlas on cardiovascular disease prevention and control. World Health Organization. 2011.2011.2011.
2. Gluckman PD, Hanson MA, Cooper C and Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med. 2008;359:61-73.
3. Wu G, Bazer FW, Cudd TA, Meininger CJ and Spencer TE. Maternal nutrition and fetal development. J Nutr. 2004;134:2169-72.
4. Roseboom TJ, van der Meulen JH, Ravelli AC, Osmond C, Barker DJ and Bleker OP. Effects of prenatal exposure to the Dutch famine on adult disease in later life: an overview. Mol Cell Endocrinol. 2001;185:93-8.
5. Cetin I, Alvino G and Cardellicchio M. Long chain fatty acids and dietary fats in fetal nutrition. J Physiol. 2009;587:3441-51.
6. Larque E, Demmelmair H, Gil-Sanchez A, Prieto-Sanchez MT, Blanco JE, Pagan A, Faber FL, Zamora S, Parrilla JJ and Koletzko B. Placental transfer of fatty acids and fetal implications. Am J Clin Nutr. 2011;94:1908S-1913S.
7. van Eijsden M, Hornstra G, van der Wal MF, Vrijkotte TG and Bonsel GJ. Maternal n-3, n-6, and trans fatty acid profile early in pregnancy and term birth weight: a prospective cohort study. Am J Clin Nutr. 2008;87:887-95.
8. Al MD, van Houwelingen AC and Hornstra G. Long-chain polyunsaturated fatty acids, pregnancy, and pregnancy outcome. Am J Clin Nutr. 2000;71:285S-91S.
9. Wolfe H. High prepregnancy body-mass index--a maternal-fetal risk factor. N Engl J Med. 1998;338:191-2.
10. Seligman LC, Duncan BB, Branchtein L, Gaio DS, Mengue SS and Schmidt MI. Obesity and gestational weight gain: cesarean delivery and labor complications. Rev Saude Publica. 2006;40:457-65.
11. Gaillard R. Maternal obesity during pregnancy and cardiovascular development and disease in the offspring. Eur J Epidemiol. 2015;30:1141-52.
12. Nelson SM, Matthews P and Poston L. Maternal metabolism and obesity: modifiable determinants of pregnancy outcome. Hum Reprod Update. 2010;16:255-75.
13. Karlsson M, Marild S, Brandberg J, Lonn L, Friberg P and Strandvik B. Serum phospholipid fatty acids, adipose tissue, and metabolic markers in obese adolescents. Obesity (Silver Spring). 2006;14:1931-9.
14. Micallef M, Munro I, Phang M and Garg M. Plasma n-3 Polyunsaturated Fatty Acids are negatively associated with obesity. Br J Nutr. 2009;102:1370-4.

15. Tomedi LE, Chang CC, Newby PK, Evans RW, Luther JF, Wisner KL and Bodnar LM. Pre-pregnancy obesity and maternal nutritional biomarker status during pregnancy: a factor analysis. Public Health Nutr. 2013;16:1414-8.
16. Whitaker RC and Dietz WH. Role of the prenatal environment in the development of obesity. J Pediatr. 1998;132:768-76.
17. Thornburg KL, Jacobson SL, Giraud GD and Morton MJ. Hemodynamic changes in pregnancy. Semin Perinatol. 2000;24:11-4.
18. Ananth CV and Basso O. Impact of pregnancy-induced hypertension on stillbirth and neonatal mortality. Epidemiology. 2010;21:118-23.
19. Xu J, Eilat-Adar S, Loria C, Goldbourt U, Howard BV, Fabsitz RR, Zephier EM, Mattil C and Lee ET. Dietary fat intake and risk of coronary heart disease: the Strong Heart Study. Am J Clin Nutr. 2006;84:894-902.
20. Wang L, Manson JE, Forman JP, Gaziano JM, Buring JE and Sesso HD. Dietary fatty acids and the risk of hypertension in middle-aged and older women. Hypertension. 2010;56:598-604.
21. Oken E, Ning Y, Rifas-Shiman SL, Rich-Edwards JW, Olsen SF and Gillman MW. Diet during pregnancy and risk of preeclampsia or gestational hypertension. Ann Epidemiol. 2007;17:663-8.
22. Mahomed K, Williams MA, King IB, Mudzamiri S and Woelk GB. Erythrocyte omega-3, omega-6 and trans fatty acids in relation to risk of preeclampsia among women delivering at Harare Maternity Hospital, Zimbabwe. Physiol Res. 2007;56:37-50.
23. Harris WS. Extending the cardiovascular benefits of omega-3 Fatty acids. Curr Atheroscler Rep. 2005;7:375-80.
24. Valensi P. Hypertension, single sugars and fatty acids. J Hum Hypertens. 2005;19 Suppl 3:S5-9.
25. Kinsella JE, Broughton KS and Whelan JW. Dietary unsaturated fatty acids: interactions and possible needs in relation to eicosanoid synthesis. J Nutr Biochem. 1990;1:123-41.
26. Wang Y, Walsh SW and Kay HH. Placental lipid peroxides and thromboxane are increased and prostacyclin is decreased in women with preeclampsia. Am J Obstet Gynecol. 1992;167:946-9.
27. Walsh SW and Wang Y. Trophoblast and placental villous core production of lipid peroxides, thromboxane, and prostacyclin in preeclampsia. J Clin Endocrinol Metab. 1995;80:1888-93.
28. Lazzarin N, Vaquero E, Exacoustos C, Bertonotti E, Romanini ME and Arduini D. Low-dose aspirin and omega-3 fatty acids improve uterine artery blood flow velocity in women with recurrent miscarriage due to impaired uterine perfusion. Fertil Steril. 2009;92:296-300.
29. Haggarty P. Fatty acid supply to the human fetus. Annu Rev Nutr. 2010;30:237-55.
30. Raj M. Obesity and cardiovascular risk in children and adolescents. Indian J Endocrinol Metab. 2012;16:13-9.
31. Freedman DS, Khan LK, Dietz WH, Srinivasan SR and Berenson GS. Relationship of childhood obesity to coronary heart disease risk factors in adulthood: the Bogalusa Heart Study. Pediatrics. 2001;108:712-8.
32. Donahue SM, Rifas-Shiman SL, Gold DR, Jouni ZE, Gillman MW and Oken E. Prenatal fatty acid status and child adiposity at age 3 y: results from a US pregnancy cohort. Am J Clin Nutr. 2011;93:780-8.
33. Ailhaud G, Massiera F, Weill P, Legrand P, Alessandri JM and Guesnet P. Temporal changes in dietary fats: role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity. Prog Lipid Res. 2006;45:203-36.
34. Standl M, Thiering E, Demmelmair H, Koletzko B and Heinrich J. Age-dependent effects of cord blood long-chain PUFA composition on BMI during the first 10 years of life. Br J Nutr. 2014;111:2024-31.

35. de Vries PS, Gielen M, Rizopoulos D, Rump P, Godschalk R, Hornstra G and Zeegers MP. Association between polyunsaturated fatty acid concentrations in maternal plasma phospholipids during pregnancy and offspring adiposity at age 7: the MEFAB cohort. Prostaglandins Leukot Essent Fatty Acids. 2014;91:81-5.
36. Hauner H, Much D, Vollhardt C, Brunner S, Schmid D, Sedlmeier EM, Heimberg E, Schuster T, Zimmermann A, Schneider KT, Bader BL and Amann-Gassner U. Effect of reducing the n-6:n-3 long-chain PUFA ratio during pregnancy and lactation on infant adipose tissue growth within the first year of life: an open-label randomized controlled trial. Am J Clin Nutr. 2012;95:383-94.
37. Shah NR and Braverman ER. Measuring adiposity in patients: the utility of body mass index (BMI), percent body fat, and leptin. PLoS One. 2012;7:e33308.
38. Sorof JM, Lai D, Turner J, Poffenbarger T and Portman RJ. Overweight, ethnicity, and the prevalence of hypertension in school-aged children. Pediatrics. 2004;113:475-82.
39. National High Blood Pressure Education Program Working Group on High Blood Pressure in C and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics. 2004;114:555-76.
40. Armitage JA, Pearce AD, Sinclair AJ, Vingrys AJ, Weisinger RS and Weisinger HS. Increased blood pressure later in life may be associated with perinatal n-3 fatty acid deficiency. Lipids. 2003;38:459-64.
41. Pinhas-Hamiel O, Lerner-Geva L, Copperman NM and Jacobson MS. Lipid and insulin levels in obese children: changes with age and puberty. Obesity (Silver Spring). 2007;15:2825-31.
42. Bremer AA, Stanhope KL, Graham JL, Cummings BP, Ampah SB, Saville BR and Havel PJ. Fish oil supplementation ameliorates fructose-induced hypertriglyceridemia and insulin resistance in adult male rhesus macaques. J Nutr. 2014;144:5-11.
43. Chechi K and Cheema SK. Maternal diet rich in saturated fats has deleterious effects on plasma lipids of mice. Exp Clin Cardiol. 2006;11:129-35.
44. Zhao JP, Levy E, Fraser WD, Julien P, Delvin E, Montoudis A, Spahis S, Garofalo C, Nuyt AM and Luo ZC. Circulating docosahexaenoic acid levels are associated with fetal insulin sensitivity. PLoS One. 2014;9:e85054.
45. Courville AB, Harel O and Lammi-Keefe CJ. Consumption of a DHA-containing functional food during pregnancy is associated with lower infant ponderal index and cord plasma insulin concentration. Br J Nutr. 2011;106:208-12.
46. Jochems SH, Gielen M, Rump P, Hornstra G and Zeegers MP. Potential programming of selected cardiometabolic risk factors at childhood by maternal polyunsaturated fatty acid availability in the MEFAB cohort. Prostaglandins Leukot Essent Fatty Acids. 2015;100:21-7.
47. Rump P, Popp-Snijders C, Heine RJ and Hornstra G. Components of the insulin resistance syndrome in seven-year-old children: relations with birth weight and the polyunsaturated fatty acid content of umbilical cord plasma phospholipids. Diabetologia. 2002;45:349-55.
48. Hornstra G. Essential fatty acids in mothers and their neonates. Am J Clin Nutr. 2000;71:1262S-9S.
49. Ailhaud G, Guesnet P and Cunnane SC. An emerging risk factor for obesity: does disequilibrium of polyunsaturated fatty acid metabolism contribute to excessive adipose tissue development? Br J Nutr. 2008;100:461-70.
50. Massiera F, Saint-Marc P, Seydoux J, Murata T, Kobayashi T, Narumiya S, Guesnet P, Amri EZ, Negrel R and Ailhaud G. Arachidonic acid and prostacyclin signaling promote adipose tissue development: a human health concern? J Lipid Res. 2003;44:271-9.

51. Shimokawa H and Vanhoutte PM. Dietary omega 3 fatty acids and endothelium-dependent relaxations in porcine coronary arteries. Am J Physiol. 1989;256:H968-73.
52. Mori TA, Watts GF, Burke V, Hilme E, Puddey IB and Beilin LJ. Differential effects of eicosapentaenoic acid and docosahexaenoic acid on vascular reactivity of the forearm microcirculation in hyperlipidemic, overweight men. Circulation. 2000;102:1264-9.
53. Morris MC, Sacks F and Rosner B. Does fish oil lower blood pressure? A meta-analysis of controlled trials. Circulation. 1993;88:523-33.
54. Cicero AF, Ertek S and Borghi C. Omega-3 polyunsaturated fatty acids: their potential role in blood pressure prevention and management. Curr Vasc Pharmacol. 2009;7:330-7.
55. Harris WS and Shearer GC. Omega-6 Fatty Acids and Cardiovascular Disease: Friend or Foe? Circulation. 2014.
56. Hashimoto M, Shinozuka K, Gamoh S, Tanabe Y, Hossain MS, Kwon YM, Hata N, Misawa Y, Kunitomo M and Masumura S. The hypotensive effect of docosahexaenoic acid is associated with the enhanced release of ATP from the caudal artery of aged rats. J Nutr. 1999;129:70-6.
57. Patterson E, Wall R, Fitzgerald GF, Ross RP and Stanton C. Health implications of high dietary omega-6 polyunsaturated Fatty acids. J Nutr Metab. 2012;2012:539426.
58. Mennitti LV, Oliveira JL, Morais CA, Estadella D, Oyama LM, Oller do Nascimento CM and Pisani LP. Type of fatty acids in maternal diets during pregnancy and/or lactation and metabolic consequences of the offspring. J Nutr Biochem. 2015;26:99-111.
59. Wang X and Chan CB. n-3 polyunsaturated fatty acids and insulin secretion. J Endocrinol. 2015;224:R97-106.
60. Kruithof CJ, Kooijman MN, van Duijn CM, Franco OH, de Jongste JC, Klaver CC, Mackenbach JP, Moll HA, Raat H, Rings EH, Rivadeneira F, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC, Wolvius EB, Hofman A and Jaddoe VW. The Generation R Study: Biobank update 2015. Eur J Epidemiol. 2014;29:911-27.
61. Nohr EA, Frydenberg M, Henriksen TB and Olsen J. Does low participation in cohort studies induce bias? Epidemiology. 2006;17:413-8.
62. Nilsen RM, Vollset SE, Gjessing HK, Skjaerven R, Melve KK, Schreuder P, Alsaker ER, Haug K, Daltveit AK and Magnus P. Self-selection and bias in a large prospective pregnancy cohort in Norway. Paediatr Perinat Epidemiol. 2009;23:597-608.
63. Rothman K. Epidemiology: An introduction. New york: Oxford university press. 2002.
64. Chen X and Wang Y. Tracking of blood pressure from childhood to adulthood: a systematic review and meta-regression analysis. Circulation. 2008;117:3171-80.
65. Juhola J, Magnussen CG, Viikari JS, Kahonen M, Hutri-Kahonen N, Jula A, Lehtimaki T, Akerblom HK, Pietikainen M, Laitinen T, Jokinen E, Taittonen L, Raitakari OT and Juonala M. Tracking of serum lipid levels, blood pressure, and body mass index from childhood to adulthood: the Cardiovascular Risk in Young Finns Study. J Pediatr. 2011;159:584-90.
66. Morrison JA, Friedman LA, Wang P and Glueck CJ. Metabolic syndrome in childhood predicts adult metabolic syndrome and type 2 diabetes mellitus 25 to 30 years later. J Pediatr. 2008;152:201-6.
67. Lucia Bergmann R, Bergmann KE, Haschke-Becher E, Richter R, Dudenhausen JW, Barclay D and Haschke F. Does maternal docosahexaenoic acid supplementation during pregnancy and lactation lower BMI in late infancy? J Perinat Med. 2007;35:295-300.

68. Stratakis N, Gielen M, Chatzi L and Zeegers MP. Effect of maternal n-3 long-chain polyunsaturated fatty acid supplementation during pregnancy and/or lactation on adiposity in childhood: a systematic review and meta-analysis of randomized controlled trials. Eur J Clin Nutr. 2014;68:1277-87.
69. Rytter D, Bech BH, Christensen JH, Schmidt EB, Henriksen TB and Olsen SF. Intake of fish oil during pregnancy and adiposity in 19-y-old offspring: follow-up on a randomized controlled trial. Am J Clin Nutr. 2011;94:701-8.

Chapter 5

Summary

Summary

Chapter 1 describes the background and hypothesis for the studies presented in this thesis. Cardio-metabolic disease is a major public health concern in adulthood. The developmental origins hypothesis proposed that cardio-metabolic disease originate during intrauterine development. This hypothesis suggests that risk factors during pregnancy may adversely affect the fetal development, and thereby increase the risk of developing diseases later in life.

Previous studies have shown that suboptimal nutrition during pregnancy is associated with higher risk of cardio-metabolic diseases. Based on these findings, it has been hypothesized that adverse exposures, acting at different stages of fetal and early postnatal development, lead to permanent adaptations in the structure, physiology and function of various organ systems. An excess or deficiency of certain fatty acids profile during pregnancy may lead to adverse consequences later in life. Especially maternal PUFAs status is required for normal growth and development. Identifying risk factors and potential mechanisms influencing the development of cardio-metabolic diseases from early life onwards is important for future preventive strategies that aim to improve cardio-metabolic health throughout the life course. Therefore, studies presented in this thesis were designed to relate maternal fatty acid levels measured in mid-pregnancy with cardio-metabolic outcomes in mothers and children.

The studies presented in this thesis were embedded in the Generation R Study, a population-based prospective cohort study from fetal life onwards in Rotterdam, The Netherlands. The Generation R Study is designed to identify early environmental and genetic determinants of growth, development and health in fetal life and childhood.

In **Chapter 2**, studies on maternal prepregnancy body mass index and gestational weight gain during pregnancy with different group of fatty acid concentrations are described. Furthermore, fatty acids during pregnancy and hemodynamic adaptations during pregnancy are also described in this chapter. In **Chapter 2.1**, we found that higher prepregnancy body mass index was associated with higher total SFA and total n-6 PUFA concentrations Higher gestational weight gain was associated, independent of prepregnancy body mass index with higher total SFA, MUFA and n-6 PUFA concentrations Thus, obesity and excessive weight gain during pregnancy might be associated with an adverse fatty acids profile. Further, we examined the associations of maternal fatty acid levels with maternal blood pressure development, umbilical and uterine artery resistance indices and the risk of gestational hypertensive disorders (**Chapter 2.2).** We observed that higher maternal SFAs, but not MUFAs, were associated with a higher blood pressure, whereas higher total n-3 PUFA and n-6 PUFA

concentrations were associated with a lower systolic and diastolic blood pressure throughout pregnancy. Higher MUFA concentrations were associated with lower placental vascular resistance indices, whereas higher n-3 and n-6 PUFAs were associated with higher placental vascular resistance indices. Only higher maternal SFA concentrations were associated with the risk of gestational hypertensive disorders. Our findings suggest that during pregnancy, adverse maternal fatty acid concentrations may lead to hemodynamic maladaptations and increased risks of gestational hypertensive disorders.

In **Chapter 3,** the associations of maternal PUFA levels during pregnancy with cardio-metabolic risk factors in childhood are described. In **Chapter 3.1,** we examined the associations of maternal PUFA concentrations during pregnancy with infant subcutaneous fat mass. Total subcutaneous fat and central-to-total subcutaneous fat ratio were calculated at 1.5, 6 and 24 months. We observed that maternal n-3 PUFA levels were associated with a higher central subcutaneous fat mass at 1.5 months, but with lower central subcutaneous fat mass at 6 months. No consistent associations were observed between maternal n-6 PUFA levels and infant subcutaneous fat mass levels. These associations were not explained by maternal socio-demographic or lifestyle related characteristics or birth characteristics. Therefore, maternal n-3 PUFA levels during pregnancy may have transient effects on infant subcutaneous fat. We also explored whether maternal PUFA concentrations during pregnancy may have persistent effects on growth and adiposity in the childhood (**Chapter 3.2**). At the age of 6 years, detailed total and abdominal body fat distribution measurements were performed using Dual-energy- X-ray absorptiometry (DXA) and abdominal ultrasound. We observed that maternal lower n-3 PUFA concentrations and higher n-6 PUFA concentrations during pregnancy are associated with a higher body fat percentage and an adverse general and abdominal fat distribution in childhood. We found the stronger associations for maternal total n-3 PUFA concentrations with childhood total body fat mass percentage, as compared to other fat measures. The associations of maternal n-3 and n-6 PUFA concentrations with detailed childhood fat mass outcomes were only partly explained by maternal and childhood characteristics. Thus, our results suggests that lower maternal n-3 PUFA concentrations and higher n-6 PUFA concentrations during pregnancy are associated with higher total body fat and abdominal fat levels in childhood.

In **Chapter 3.3**, we assessed the associations of maternal PUFA concentrations during pregnancy with childhood blood pressure. At the age of 6 years systolic and diastolic blood pressure was measured four times with one-minute intervals using a validated automatic sphygmanometer. We found that higher maternal n-3 PUFA and lower n-6 PUFAwt% during pregnancy are associated with a lower systolic blood

pressure in childhood. These associations were only partly explained by maternal and childhood characteristics. Thus, our results suggest that maternal intake of PUFAs during pregnancy is important for fetal and infant development. In **Chapter 3.4**, we examined whether maternal plasma n-3 and n-6 PUFA levels during pregnancy are associated with childhood lipids and insulin levels. We observed that after adjustment for potential confounders, higher maternal total n-3 PUFAs and specifically docosahexaenoic acid levels during pregnancy are associated with higher childhood total-cholesterol, HDL-cholesterol and insulin levels. Only individual maternal n-6 PUFAs, not total maternal n-6 PUFA levels, tended to be associated with childhood lipids and insulin levels. These results were not materially affected by additional adjustment for childhood body mass index. Our results suggest that maternal PUFAs status during pregnancy might influence offspring lipids and insulin metabolism.

In **Chapter 4** we provide a general discussion in which the studies described in this thesis are described in broader context, and implications and suggestions for future research are discussed.

In conclusion, findings from this thesis suggest that maternal fatty acid levels during pregnancy are associated with maternal and childhood cardio-metabolic outcomes. Although the observed associations were relatively small to moderate, they may be important for cardio-metabolic disease on a population level. Based on our finding, adequate maternal fatty acids profile during pregnancy is important for maternal and child health. Cardio-metabolic prevention strategies should start as early in life as possible and should focus on maternal nutritional status during pregnancy by improving fatty acids profile.

Samenvatting

Hoofdstuk 1 beschrijft de achtergrond en hypothese voor de studies beschreven in dit proefschrift. Cardiometabole ziekten vormen een belangrijk volksgezondheidsprobleem voor volwassenen. De 'Developmental Origins' hypothese stelt dat de grondslag van deze ziekten al ligt tijdens de vroege ontwikkeling. Volgens deze hypothese zou blootstelling aan bepaalde factoren tijdens de zwangerschap de ontwikkeling van de foetus op een ongunstige manier beïnvloeden, en daarmee het risico op ziekten op latere leeftijd te verhogen. Eerdere studies hebben laten zien dat suboptimale voeding tijdens de zwangerschap is geassocieerd met een hoger risico op cardiometabole ziekten. Gebaseerd op deze resultaten is de hypothese gesteld dat blootstelling aan bepaalde ongunstige factoren tijdens verschillende stadia van de foetale en vroeg postnatale ontwikkeling, leidt tot blijvende aanpassingen in de structuur, fysiologie en functie van verschillende orgaansystemen. Een teveel of tekort aan bepaalde vetten (vetzuren) tijdens de zwangerschap zou nadelige gevolgen kunnen hebben in het latere leven. Vooral een optimale meervoudig onverzadigde vetzuur status van de moeder is belangrijk voor een normale groei en ontwikkeling van haar kind. Het identificeren van vroege risicofactoren en potentiële mechanismen die het ontstaan van cardiometabole ziekten beïnvloeden is belangrijk voor de ontwikkeling van toekomstige preventieve strategieën om de cardiometabole gezondheid tijdens de gehele levensloop te verbeteren. Het doel van de studies gepresenteerd in dit proefschrift was dan ook om maternale vetzuurconcentraties, gemeten tijdens het tweede trimester van de zwangerschap, te relateren aan cardiometabole gezondheidsuitkomsten in zowel moeders als hun kinderen.

De studies die beschreven worden in dit proefschrift maken deel uit van het Generation R onderzoek, een populatie-gebaseerd prospectief cohortonderzoek, in Rotterdam, Nederland, waarin zwangere vrouwen en hun kinderen worden gevolgd. Het Generation R onderzoek is opgezet om vroege omgevings- en genetische factoren te identificeren die van invloed zijn van op de groei, ontwikkeling en gezondheid tijdens het foetale leven en tijdens de kindertijd.

In **hoofdstuk 2** worden studies beschreven naar de relaties tussen maternale body mass index voor de zwangerschap, gewichtstoename tijdens de zwangerschap en de verschillende groepen vetzuren. Daarnaast beschrijft dit hoofdstuk de invloed van vetzuren op hemodynamische aanpassingen tijdens de zwangerschap. In **hoofdstuk 2.1** hebben we aangetoond dat een hoger body mass index geassocieerd is met hogere totale verzadigde vetzuurconcentraties en hogere totale omega-6 meervoudig onverzadigde vetzuurconcentraties. Een snelle gewichtstoename was geassocieerd met hogere totale verzadigde, enkelvoudig onverzadigde en omega-6 meervoudig onver-

zadigde vetzuurconcentraties. Obesitas en een verhoogde gewichtstoename tijdens de zwangerschap zijn dus mogelijk geassocieerd met een nadelig vetzuur profiel. Ook hebben we de associaties van maternale vetzuurconcentraties met maternale bloeddruk ontwikkeling, de vaatweerstand van de arteria umbilicalis en de arteria uterina en het risico op pre-eclampsie en zwangerschapshypertensie onderzocht (**hoofdstuk 2.2**). We zagen dat hogere maternale verzadigde vetzuurconcentraties, maar niet meervoudig onverzadigde verzuurconcentraties, geassocieerd waren met een hogere bloeddruk, en dat hogere totale omega-3 meervoudig onverzadigde vetzuurconcentraties en omega-6 meervoudig onverzadigde vetzuurconcentraties daarentegen zijn geassocieerd met een lagere systolische en diastolische bloeddruk tijdens de zwangerschap. Hogere verzadigde vetzuurconcentraties waren geassocieerd met een lagere placentaire vaatweerstand, hogere omega-3 en omega-6 meervoudig onverzadigde vetzuren waren geassocieerd met een hogere placentaire vaatweerstand. Alleen hogere maternale verzadigde vetzuurconcentraties waren geassocieerd met het risico op pre-eclampsie of zwangerschapshypertensie. Onze resultaten suggereren dat suboptimale verzuurconcentraties tijdens de zwangerschap, gekarakteriseerd door hogere concentraties SFA en lagere concentraties PUFA, kunnen leiden tot ongunstige hemodynamische aanpassingen en een verhoogd risico op pre-eclampsie of zwangerschapshypertensie.

In **hoofdstuk 3** beschrijven we de associaties van maternale meervoudig onverzadigde vetzuurconcentraties met verschillende risicofactoren voor cardiometabole ziekten bij het kind. In **hoofdstuk 3.1** hebben we gekeken naar de associaties van maternale meervoudig onverzadigde vetzuurconcentraties tijdens de zwangerschap met subcutane vetmassa op peuterleeftijd. Totaal subcutaan vet en centraal-totale vetmassa ratio zijn bepaald op de leeftijden van 1.5, 6 en 24 maanden. We zagen dat maternale omega-3 meervoudig onverzadigde vetzuurconcentraties geassocieerd waren met een hogere centrale subcutane vetmassa op de leeftijd van 1.5 maanden, maar met een lagere centrale vetmassa op de leeftijd van 6 maanden. We zagen geen consistente associaties tussen maternale omega-6 onverzadigde vetzuurconcentraties en subcutane vetmassa in de peutertijd. De gevonden associaties werden niet verklaard door verschillen in sociaaleconomische en leefstijl gerelateerde kenmerken. Maternale omega-3 meervoudig onverzadigde vetzuurconcentraties zouden daarom mogelijk tijdelijke effecten kunnen hebben op de subcutane vetmassa tijdens de peutertijd. Ook hebben we onderzocht of maternale meervoudig onverzadigde vetzuurconcentraties blijvende invloed hebben op de groei en de vetverdeling van het kind (**hoofdstuk 3.2**). Op de leeftijd van 6 jaar zijn gedetailleerde metingen van de totale en abdominale vetmassa gedaan met behulp van Dual-energy X-ray absorptiometry (DXA) scans en abdominale echografie. We zagen dat lagere maternale

omega-3 meervoudig onverzadigde verzuurconcentraties en hogere omega-6 onverzadigde vetzuurconcentraties tijdens de zwangerschap geassocieerd waren met een hoger percentage lichaamsvet en een ongunstige totale en abdominale vetverdeling bij het kind. De associaties waren het sterkst voor het totale percentage lichaamsvet. De gevonden associaties werden deels verklaard door verschillen in andere kenmerken van moeder en haar kind. Deze bevindingen suggereren dat lagere maternale omega-3 en hogere omega-6 meervoudig onverzadigde vetzuurconcentraties tijdens de zwangerschap zijn geassocieerd met hoger totaal lichaamsvet en abdominaal vet bij kinderen.

In **hoofdstuk 3.3** hebben we gekeken naar de associaties van maternale meervoudig onverzadigde vetzuurconcentraties tijdens de zwangerschap met de bloeddruk van het kind. Op de leeftijd van 6 jaar zijn systolische en diastolische bloeddruk vier keer gemeten met tijdsintervallen van 1 minuut met behulp van een gevalideerde automatische sphygmanometer. We zagen dat hogere maternale omega-3 meervoudig onverzadigde vetzuurconcentraties en lagere omega-6 meervoudig onverzadigde verzuurconcentraties tijdens de zwangerschap zijn geassocieerd met een lagere systolische bloeddruk op 6-jarige leeftijd. De gevonden associaties werden maar deels verklaard door verschillen in andere kenmerken van moeder en kind. Deze resultaten suggereren dat maternale inname van meervoudig onverzadigde vetzuren tijdens de zwangerschap belangrijk is voor de ontwikkeling van het kind, zowel tijdens het foetale leven als tijdens de peutertijd. In **hoofdstuk 3.4** hebben we onderzocht of maternale omega-3 en omega-6 meervoudig onverzadigde vetzuurconcentraties gerelateerd zijn aan lipiden- en insuline concentraties bij haar kind. Na correctie voor potentiele confounders waren hogere maternale totale omega-3 meervoudig onverzadigde vetzuren, en vooral docosahexaeenzuur, tijdens de zwangerschap geassocieerd met hogere totaal cholesterol, HDL-cholesterol en insuline concentraties bij het kind. Individuele omega-6 meervoudig onverzadigde vetzuurconcentraties, maar niet totale omega-6 concentraties, waren geassocieerd met lipiden- en insuline concentraties. Deze resultaten veranderden niet na extra correctie voor het body mass index van het kind. Onze resultaten suggereren dat maternale meervoudig onverzadigde vetzuurstatus mogelijk invloed heeft op de lipiden- en insuline metabolisme van het kind.

In **hoofdstuk 4** geven we een algemene discussie waarin de studies beschreven in dit proefschrift worden beschouwd in een bredere context, en implicaties en suggesties voor toekomstig onderzoek gegeven.

Concluderend, de bevindingen van dit proefschrift suggereren dat maternale vetzuurconcentraties tijdens de zwangerschap zijn geassocieerd met cardiometabole gezondheidsuitkomsten bij zowel de moeder als haar kind. Hoewel de gevonden ef-

fecten relatief klein zijn, zijn deze mogelijk van belang op populatie niveau. Op basis van onze bevindingen blijkt een optimaal maternaal vetzuurprofiel van belang voor de gezondheid van zowel de moeder als haar kind. Preventieve strategieën zouden daarom al zo vroeg mogelijk in het leven moeten starten en zouden zich moeten richten op het verbeteren van het vetzuurprofiel van de vrouw tijdens de zwangerschap.

Chapter 6

Abbreviations
Authors' affiliations
Publication list
About the author
PhD portfolio
Words of gratitude

Abbreviations

AA	arachidonic acid
ALA	alpha-linolenic acid
ANOVA	analysis of variance
AvsG	android/gynoid fat mass ratio
BMI	body mass index
CI	confidence interval
DGLA	dihomo-γ-linolenic acid
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
DTA	docosatetraenoic acid
DXA	dual-energy X-ray absorptiometry
EPA	eicosapentaenoic acid
FFQ	food-frequency questionnaire
GLA	γ-linoleic acid
GWG	gestational weight gain
HDL	high-density lipoprotein
IOM	institute of medicine
LA	linoleic acid
LDL	low-density lipoprotein
MUFA	monounsaturated fatty acids
No	number of participants
OR	odds ratio
PPAREA	abdominal preperitoneal fat mass
PUFA	polyunsaturated fatty acid
RCT	randomized controlled trial
SD	standard deviation
SDS	standard deviation score
SFA	saturated fatty acid
SPSS	statistical package for the social sciences
TFM	total fat mass
wk	weeks
wt%	weight percentage
y	year

Affiliations

Department of Child and Adolescent Psychiatry, Erasmus MC, Rotterdam, The Netherlands
H Tiemeier

Department of Epidemiology, Erasmus MC, Rotterdam, The Netherlands
JF Felix, R Gaillard, O Gishti, A Hofman, VWV Jaddoe, H Tiemeier, AJ Vidakovic, T Voortman

EPI-Unit, Institute of Public Health, University of Porto, Porto, Portugal
S Santos

Harvard School of Public Health, Boston, MA, USA
MA Williams

Ludwig-Maximilians-Universität München, Division of Metabolic and Nutritional Medicine, Dr. von Hauner Children's Hospital, University of Munich Medical Centre, München, Germany
H Demmelmair, B Koletzko

Department of Obstetrics and Gynecology, Erasmus MC, Rotterdam, The Netherlands
EAP Steegers

Department of Pediatrics, Erasmus MC, Rotterdam, The Netherlands
L Duijts, R Gaillard, O Gishti, VWV Jaddoe, AJ Vidakovic

The Generation R Study Group, Erasmus MC, Rotterdam, The Netherlands
JF Felix, R Gaillard, O Gishti, VWV Jaddoe, S Santos, H Tiemeier, AJ Vidakovic, T Voortman

Publications

1. **Vidakovic AJ**, Jaddoe VW, Gishti O, Felix JF, Williams MA, Hofman A, Demmelmair H, Koletzko B, Tiemeier H, Gaillard R. Body mass index, gestational weight gain and fatty acid concentrations during pregnancy: the Generation R Study. Eur J Epidemiol. 2015;30(11):1175-85.

2. **Vidakovic AJ**, Jaddoe VW, Steegers EA, Gaillard R. Maternal fatty acid profiles, hemodynamic adaptations and gestational hypertensive disorders. The Generation R Study. Submitted

3. **Vidakovic AJ**, S Santos, Williams MA, Duijts L, Hofman A, Demmelmair H, Koletzko B, Jaddoe VW, Gaillard R. Maternal plasma n-3 and n-6 polyunsaturated fatty acid concentrations during pregnancy and subcutaneous fat mass in infancy. Obesity (Silver Spring). 2016;24(8):1759-66.

4. **Vidakovic AJ**, Gishti O, Voortman T, Felix JF, Williams MA, Hofman A, Demmelmair H, Koletzko B, Tiemeier H, Jaddoe VW, Gaillard R. Maternal plasma PUFA concentrations during pregnancy and childhood adiposity: the Generation R Study. Am J Clin Nutr. 2016;103(4):1017-25.

5. **Vidakovic AJ**, Gishti O, Steenweg-de Graaff J, Williams MA, Duijts L, Felix JF, Hofman A, Tiemeier H, Jaddoe VW, Gaillard R. Higher maternal plasma n-3 PUFA and lower n-6 PUFA concentrations in pregnancy are associated with lower childhood systolic blood pressure. J Nutr. 2015;145(10):2362-8.

6. **Vidakovic AJ**, Jaddoe VW, Demmelmair H, Koletzko B, Gaillard R. Maternal plasma polyunsaturated fatty acid levels during pregnancy and childhood lipids and insulin levels. Nutr Metab Cardiovasc Dis. In press.

About the author

Aleksandra Jelena Vidakovic was born in Zvornik, Serbia, on June 4th, 1985. She graduated from medical high school in 2005 in Bijeljina. In 2013, she obtained her MD degree at the University of Belgrade, Serbia. In 2011/2012 she volunteered at the Clinical for Pulmonary Diseases of the Clinical Centre of Serbia in Belgrade where she conducted research project entitled 'Spirometry and Cat Questionnaire as a Comparative Method for Assessment of Severity of Chronic Obstructive Pulmonary Disease' under supervision of Prof.dr. M.Mitic-Milikic. In 2013, she came to Erasmus Medical Center on an ERAWEB scholarship, and in 2014 she obtained a Master of Science degree, specialisation Clinical Epidemiology, at the Netherlands Institute for Health Sciences. In 2014, she continued her studies with a Doctor of Science degree in Clinical Epidemiology and a PhD project entitled 'Fatty Acids during Pregnancy and Cardio-metabolic Outcomes in Mothers and their Children' under supervision of Prof.dr. V.W.V. Jaddoe (Departments of Epidemiology and Pediatrics) and Prof.dr. E.A.P. Steegers (Department of Obstetrics and Gynaecology). The results of this work are presented in this dissertation. After obtaining her PhD she will go back to Serbia where she would like to start the residency in Paediatrics.

Portfolio

Summary PhD activities

Name PhD student:	Aleksandra Jelena Vidakovic
Erasmus MC Department:	Clinical Epidemiology
Research School:	Netherlands Institute for Health Sciences
PhD period:	August 2013 – November 2016
Promotors:	Prof.dr. V.W.V. Jaddoe, Prof.dr. E.A.P. Steegers

1. PhD training	Year	Workload (ECTS)
General courses		
Master's degree in Health Sciences, specialization Clinical Epidemiology, NIHES, Erasmus University Rotterdam, the Netherlands	2013-2014	
Doctor of Science's degree in Health Sciences, specialization Clinical Epidemiology, NIHES, Erasmus University Rotterdam, the Netherlands	2014-2015	
Principles of research in medicine		0.7
Clinical decision analyses		0.7
Methods of public health research		0.7
Health economics		0.7
Genome wide association analysis		1.4
Genomics in molecular medicine		1.4
Markers and prognostic research		0.7
The practice of epidemiologic analysis		0.7
Conceptual foundation of epidemiologic study design		0.7
Causal inference		0.7
History of epidemiologic ideas		0.7
Advances in epidemiologic analysis		0.4
Causal mediation analysis		0.7
Study Design		4.3
Biostatistical methods 1: Basic principales		5.7
Biostatistical methods 2: Classical regression models		4.3
Clinical epidemiology		5.7
Methodologic topics in epidemiological research		1.4
Advanced courses		
Missing values in clinical research		0.7
Women's health		0.9
Principles of epidemiologic data-analysis		0.7
Public health in low and middle income countries		3.0
Courses for the quantitative researcher		1.4
Maternal and child health		0.9
Bayesian statistics		1.4
Planning and evaluation of screening		1.4
Health services: research and practice		0.9
Psychology in medicine		1.4
Introduction to medical writing		1.1

General academic skills		
Scientific Writing in English for Publication, Erasmus MC, the Netherlands		2.0
Development research proposal		2.5
Oral research presentation		1.4
Seminars and workshops		
Generation R Research meetings, Erasmus MC, The Netherlands	2013-2016	1.0
Seminars at the department of Epidemiology, Erasmus MC, The Netherlands	2013-2016	1.0
(Inter)national congresses and presentations		
Developmental Origins of Health and Disease (DOHaD), Cape Town. Oral presentation	2015	1.4
Scholarships and grants		
Eraweb Master student grant	2013-2014	
Eraweb PhD student grant	2013-2016	
Vereniging Trustfonds Erasmus Universiteit Rotterdam, travel grant	2015	

Words of gratitude

I would like to express my gratitude to everyone who has helped me in the preparation of this thesis without whom the publication of this thesis would not have been possible.

My sincere appreciation is extended to the Generation R team for all the effort that they put in the realization of this project. Dear parents and kids, thank you for your participation in this study which could not have been completed without you.

Special thanks go to my promotors, Prof.dr. Jaddoe and Prof.dr. Steegers. Dear Vincent, thank you so much for all your support, advice, guidance and patience that you have given me over the past three years. Thank you for showing me that the authority and respect can only be acquired through knowledge and correct attitude towards students. When I first came to the Generation R department as a Master student, we decided to write a paper focused on fatty acids as an exposure. That work was quite a challenge for me since I was not familiar with this topic. But with your trust and support I managed to overcome all difficulties and I even grew some love for the topic itself. During these three years I have had the opportunity to learn a lot from you. You were always prompt with your answers, comments and suggestions about my work and maybe more importantly, you were willing to give me your support when I needed it the most. I also need to thank you for teaching me how to recognize the opportunities and how to prioritize work instead of dissipating it on too many of them. I really hope that we will share other challenges in the future.

Dear Eric, thank you for sharing your thoughts and suggestions which have significantly improved this thesis. I have definitely learned a lot from your comments and remarks which added to my work a more insightful perspective on gynaecology-related issues.

A person who has always been there for me through all my ups and downs is my co-promotor Romy. Dear Romy, our delightful cooperation that started on 16.12.2013 has now come to an end. I am really happy and grateful to have had the opportunity to work with such a great person like you. I cannot thank you enough for all your contribution to this work and all the succinct explanations you have given me. I wish you all the best in your future career (especially in your upcoming period) and your private life as well.

Dear committee members, Prof.dr. Reiss, Prof.dr. van Rossum and Prof.dr. Smit, thank you for agreeing to read and evaluate my thesis. It is an immense honor to me, to have you as the members of the reading committee. I also kindly thank Prof.dr. van der Beek, Prof.dr. Franco and Prof.dr. J.C. de Jongste for agreeing to be the members of the plenary committee during my PhD defense.

I would also like to extend my appreciation to Prof.dr. Koletzko and Dr. Demmelmair for the time and effort that they have put into this work. Your help and useful suggestions have definitely improved the overall quality of my thesis.

I would like to thank all my co-authors: Prof.dr. Hofman, Prof. Williams, Prof.dr. Tiemeier, Dr. Felix, Dr. Duijts, Dr. Gishti, MSc. Santos, Dr. Steenweg-de Graaff and Dr. Voortman, for their helpful comments and pleasant collaboration.

I owe a special thanks to ERAWEB team, Prof.dr. Hofman for being responsible for this nice initiative that allowed me and other students to do research in high quality institutions. Thank you Astrid, Monique and Lenie for your support during my PhD project in this lovely country.

A special word of thanks goes to Olta from whom I have learned a lot. Dear Olta, thank you for your support during Romy's absence, but also afterwards. You are really a great person, always able to help the others and I appreciate that I could work with you. I wish you all the best in the future.

A special thanks to all my friends and colleagues who have given me immense support and love during my stay in the Netherlands. Dear Blin and Juan Carlos, words cannot express how much I appreciate the time we spent together during our Master programme. Even though we are apart now, you will always be in my heart. Milane, Nevena, Majo, Jelena, Kozeta, Sara, Bruna, Adela, Ivana, Desi, Nikola, thank you for all the amazing moments we shared. Sunayna, thank you for your support and love and for helping me adapt to new things and situations. Patricia, Claudia, Rose and Ronald, thank you for your support and help in the past three years. Special thanks to my office mates: Zoe, Tim and Martijn. Guys, working with you this thesis have become much more than just a work of science. Tim, without your help with my PhD motivational letter, I probably would not be writing this now. Zoe, you taught me how to work with music and you always made us laugh with your video clips. Martijn, your positive attitude and humour definitely made our days and kept us going. I will miss you a lot. Many thanks to Laura, Kozeta, Claire, Willem, Strahinja, Bruna, Ryan, Suzanne, Elise, Florianne, Marleen, Sanne, Mirjana, Tamara, Gavro, Marjolein, Evelien, Niels, Philip, Liza, Ivonne, Carlijn, Lealine, Guannan, Hanneeke, Fadila. Thank you for being great colleagues in the past few years. I really hope we will meet in the future and if you ever come to Serbia, my door is always open for you.

A huge thanks goes to my girls, my gorgeous paranymphs: Susana and Ellis. I am glad that you are part of my life and I cannot thank you enough for all your help and all the sad and happy moments we have shared. Our friendship will not end with my leaving and you will always be a part of me.

Boki, Dragana, Dejane, Antigoni, Milane,teta Rado, Jelena, Olja, Dragana, thank you for becoming a part of my life and for making my stay in this country even more

beautiful. I want to give my special thanks to Father Voja who was always patient enough to listen to me in all my moods. He was encouraging and helpful just like a real father. Ljiko, Djidjo, Andrea, Jeco, Vanja, Ivana, Mico, Lido, Sanjo, Ana, this thesis would not be finished without your guidance and love. Thank you for every single text message, phone call and word of support. Milka, Vaso, Dule, Maro, Ivo, thank you for all your spiritual support and love that you have given me. I owe a special thanks to my godfather and his family and also to my family that has always pushed me to go further and helped me to never give up. Gogo, thank you a lot for always being there for me. You made every step of the way a bit easier.

My deepest gratitude goes to my mother for her unflagging love and support throughout my life. Dear mom, thank you for making my stay in this country easier. Thank you for always being there for me; my support, my joy and the sweetest person of my life. Your love, your visits and your sacrifice are the reason I am here now. This triumph is yours as much as it is mine. Волим те МАМА!

“Well, maybe it started that way. As a dream, but doesn’t everything. Those buildings. These lights. This whole city. Somebody had to dream about it first. And maybe that is what I did. I dreamed about coming here, but then I did it.”

Roald Dahl, James and the Giant Peach