ETHNICITY, NUTRITION, AND PREGNANCY:

FOOD FOR THOUGHT

Ethnicity, nutrition, and pregnancy: food for thought
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ETHNICITY, NUTRITION, AND PREGNANCY:

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Als je doel Ithaka is en je vertrekt daarheen,
dan hoop ik dat je tocht lang zal zijn,
en vol nieuwe kennis, vol avontuur.

Vrees geen Laistrigonen en Kyclopen, of een woedende Poseidon;
je zult ze niet tegenkomen op je weg, als
je gedachten verheven zijn, en emotie
je lichaam en geest niet verlaat.
Laistrigonen en Kyclopen, en de razende Poseidon
zul je niet tegenkomen op je weg,
als je ze al niet meedroeg in je ziel, en
je ziel ze niet voor je voeten werpt

Ik hoop dat je tocht lang mag zijn,
de zomerochtenden talrijk zijn, en
dat het zien van de eerste havens
je een ongekende vreugde geeft.
Ga naar de warenhuizen van Fenicië,
neem er het beste uit mee.
Ga naar de steden van Egypte, en
leer van een volk dat ons zoveel te leren heeft.

Verlies Ithaka niet uit het oog;
daar aankomen was je doel.
Maar haast je stappen niet;
Het is beter dat je tocht duurt en duurt
en je schip pas ankert bij Ithaka,
wanneer je rijk geworden bent
van wat je op je weg hebt geleerd.

Verwacht niet dat Ithaka je meer rijkdom geeft.
Ithaka gaf je een prachtige reis;
zonder Ithaka zou je nooit vertrokken zijn.
Het gaf je alles al, meer geven kan het niet.

En mocht je vinden dat Ithaka arm is,
denk dan niet dat het je bedroog.
Want je bent een wijze geworden, hebt intens geleefd,
en dat is de betekenis van Ithaka.

Konstantinos Kavafis (1863-1933)

Contents

Chapter 1

General introduction

Ethnicity, nutrition, and pregnancy: a background

In the late 1980s, David Barker (an epidemiologist in Southampton, UK) and his colleagues made an observation that was intriguing at the time: they observed that in individuals born weighing less than 2.5 kg the risk of death from coronary heart disease was twice that of individuals born weighing 4.0 kg or more.[1,2] Their observations were a first step in the development of what is now known as the "fetal origins of disease" hypothesis: that certain prenatal factors influence the intrauterine environment in a way that affects not only fetal growth, but also the propensity to develop disease in later life.[3,4] Nowadays, this hypothesis is the basis of many epidemiologic birth cohort studies throughout the world, and marks the accepted relevance of birth weight not only as an outcome measure (reflecting health in pregnancy), but also as a strong risk indicator of children's future health.

Worldwide, large differences exist in the birth weight distribution of ethnically diverse populations, with the lowest birth weights and highest proportion of growth-restricted infants usually found among minority populations, such as the African/Negroid groups in the US[5-10] and the Asian populations in the UK[11-13] Also in the Netherlands, considerable disparities in birth weight have been reported among infants of Surinamese, Turkish, Moroccan, and Dutch origin.[14-17] From a preventive point of view, insight into the prenatal factors that influence fetal growth is necessary in order to explain and adequately address these ethnic disparities. However, such insight is difficult to obtain; it is even unknown whether the association between birth weight and short- and long-term outcomes is the same across ethnic groups.

The fetal (intrauterine) environment is influenced by a multiplicity of factors,[18] which differ in origin, pathway of effect, and most importantly, modifiability. These factors include what may be called "environmental" determinants, factors such as maternal smoking, psychosocial stress, and nutrition, which are all to some degree modifiable, as well as "constitutional" determinants, (largely) unmodifiable factors that influence fetal growth in a more natural way, either genetically (e.g., fetal sex) or nongenetically (e.g., maternal age).[19] Interestingly, the worldwide ethnic disparities in birth weight coincide with observations that women from these minority groups (e.g., African or Asian) often consume lower-quality diets than women of European/Caucasian origin,[20-25] which raises the question: are differences in maternal nutrition the key to explaining ethnicity-related differences in birth weight?

During pregnancy, virtually all nutrients are essential to the fetus, as they are either structural components of body tissues (e.g., essential fatty acids in cell membranes) or are involved in the many metabolic or growth-related processes (e.g., folate for cell division).[26,27] Predominantly through the placenta, the mother supplies the required nutritional components. An inadequate supply may result in general growth restriction or specific developmental and growth disorders (e.g., neural tube defects). Indeed, observational studies have described associations between

a poor maternal nutritional status and fetal growth restriction or other abnormalities for almost every nutrient.[18,28,29] However, experimental evidence is less conclusive.

Theoretically, experimental studies have the power of providing causal proof, but for good reason there is limited scope for these types of studies in humans. Studies that have been conducted (mostly in developing countries) often suffer from methodological constraints, including generalizability: what may be true under extreme nutritional conditions may not resemble relationships that exist when there is normal variability of nutrition (in developed countries). Other frequently occurring methodological problems include inadequate sample size and randomization, lack of data on maternal nutrient status and changes to this, uncertain compliance, and large losses to follow-up, while at the same time analysis is often compromised by inadequate control for confounding factors.[28,30] Yet, the most important constraint of both experimental and observational studies is perhaps their time frame, as the majority of studies have been conducted during mid- to late pregnancy.[31,32] Current knowledge pinpoints the fetal growth trajectory as being set very early in gestation;[33,34] the time frame from mid- to late gestation may thus be too late for observing nutrient effects, either detrimental or beneficial.[31] As a result, we still know relatively little about optimal maternal nutrition for fetal growth and development, and consequently, about the role this factor plays in explaining ethnic disparities in fetal growth.

Aim of this thesis

The present thesis aims to elucidate the role of maternal nutrition in explaining ethnicity-related differences in fetal growth, as measured by birth weight at term (≥37.0 weeks' gestation) and prevalence of small-for-gestational-age (SGA) births. For this purpose, this thesis examines (1) the association between ethnicity and maternal nutrition (and determinants thereof); (2) the association between ethnicity and birth weight (focusing on explanatory factors other than nutrition); and (3) the role of maternal nutrition as a determinant of birth weight. The studies described in this thesis were embedded in a large, prospective cohort study in Amsterdam, the Netherlands: the Amsterdam Born Children and their Development (ABCD) study. In (1) and (3), the focus will be on two specific nutritional factors, or to be more precise, one specific micronutrient: folate/folic acid (the latter being the synthetic form of the vitamin, found in dietary supplements and fortified foods) and a specific group of nutrients, the n-3 and n-6 essential fatty acids and their derivatives (the long-chain polyunsaturated fatty acids, LC-PUFAs).

The remainder of this chapter describes the general design of the ABCD study and provides some background information on the nutrients of interest. The chapter concludes with the research questions addressed in this thesis.

ABCD study: general design

To gain more insight into the influence of prenatal factors on children's health at birth as well as in later life, and to specifically investigate the ethnic heterogeneity in both these risk factors and health outcomes, a prospective cohort study – the ABCD study – was initiated in 2003 by the Municipal Health Service Amsterdam*, in cooperation with the Academic Medical Center (www.abcd-study.nl). Between January 2003 and March 2004, all pregnant women in Amsterdam were asked to participate in the ABCD study during their first prenatal visit to an obstetric care provider (general practitioner, midwife, or hospital gynecologist). This approach allowed for the inclusion of pregnant women from all of the main ethnic groups in Amsterdam: Dutch, Surinamese, Antillean (including Aruba), Turkish, Moroccan, and Ghanaian.[35] Moreover, it allowed for the inclusion of pregnant women from early pregnancy onwards, as the first prenatal visit generally takes place at 12 weeks' gestation.

For all of the women approached, the care provider completed a registration form with personal data such as name, address, and date of birth. Based on this information, a questionnaire covering sociodemographic characteristics, obstetric history, lifestyles, and psychosocial conditions was sent to the pregnant woman's home address within two weeks; and the women were requested to return it to the Municipal Health Service by prepaid mail. In addition, women were invited to participate in the ABCD biomarker study (the details of which will be given in the next section). The pregnancy questionnaire was in Dutch, but was accompanied by an English, Turkish, or Arabic translation depending on the woman's country of birth. Turkish- and Arabic-speaking women who had reading difficulties or were illiterate were invited to contact one of a group dedicated, trained female interviewers for oral administration of the questionnaire. With these supportive measures, the study group enhanced the participation of all women, regardless of ethnic origin, educational level, or Dutch language proficiency.

Finally, information on pregnancy outcomes was obtained via the Youth Health Care department of the Municipal Health Service. According to law, all children born in Amsterdam after 24 weeks' gestation (either stillborn or liveborn) must be registered at the municipality's Registry Office, after which a "mutation report" is sent to Youth Health Care. Between the 4th and 7th day after delivery, nurses from this department visit the liveborn infants and their parents to screen for congenital disorders. At this time they also record the date of delivery, infant sex, birth weight, and gestational age [based on ultrasound or, if unavailable (<10%), on the timing of the last menstrual period] as provided by the obstetric care provider.

* *Until May 2005, the Public Health Service of Amsterdam was known as the Municipal Health Service. For the purpose of consistency, we have adhered to the term Municipal Health Service throughout this manuscript.*

Maternal nutrition in the ABCD study

In the pregnancy questionnaire of the ABCD study, maternal nutrition was addressed with two specific questions: one regarding the use of folic acid supplements before or during pregnancy, and another regarding the consumption of fish and fish oil as a major source of n-3 LC-PUFAs. In addition, maternal nutrient status, including but not limited to n-3 and n-6 fatty acids and folate, was measured in blood. Biochemical analyses generally allow for a more objective measure of the dietary intake than can be obtained by estimates from an extensive food frequency questionnaire alone,[36,37] and have the advantage of not suffering from culture-specific information bias, a desirable feature for the ABCD study.

For this additional biomarker study, women were invited to donate an extra blood sample (10 mL EDTA and 10 mL serum) during the routine blood collection for screening purposes following the first antenatal check-up. The samples were subsequently sent to the Regional Laboratory of Amsterdam (either by courier or overnight mail), where they were processed and stored at −80°C until analysis. Unavoidably, this pragmatic approach to blood collection introduced the limitation of a delay in time-to-processing, with potential consequences for the validity of the measurements; this issue will be addressed in a separate chapter of this thesis.

In total, nine nutrients/nutrient groups were analyzed in maternal serum and plasma samples: the minerals iron, zinc, magnesium and calcium, the vitamins A, D, B12, and folate and the n-3 and n-6 essential fatty acid families. As described above, the focus of this thesis will be on folate/folic acid and the n-3 and n-6 fatty acids.

Selected maternal nutrients: folate

The B vitamin folate is critically important for fetal development because of its role in DNA synthesis and cell division.[38,39] In the 1990s it became evident that folic acid supplementation in the preconception period prevents neural tube defects. Since then, worldwide campaigns and, in some countries, fortification policies have been introduced to promote the intake of folic acid supplements and food folate.[39,40]

A number of studies have investigated the effect of folate on fetal growth measures, such as birth weight and being small for gestational age. However, as it is for the majority of nutrients, the evidence is inconclusive. While observational studies have shown lower blood folate levels and higher homocysteine levels (as a marker of folate deficiency)[41] in mothers of growth-restricted infants,[38,42-47] supplementation studies have yielded mixed results.[48] Nevertheless, a preventive effect of higher folate intakes has been shown particularly in women at high risk of folate deficiency.[49,50] In 2001, it was suggested that especially women with closely spaced pregnancies are at high risk of folate deficiency in the subsequent pregnancy, and that this deficiency contributes to the excess risk of fetal growth restriction associated with short interpregnancy intervals.[51] Consequently, folic acid supplementation may be of particular importance in preventing fetal growth restriction in this group.

In 2004, it was shown that despite many efforts to promote folic acid supplementation, folic acid use in the majority of countries is still low: most countries reported intake rates below 50%.[52] In the Netherlands, a recent study found that although 74% of the women used folic acid at some time during pregnancy, only 43% used it during the entire recommended period (from 4 weeks before until 8 weeks after conception).[53] Few studies have ventured to touch upon ethnic differences in folic acid use, but their results suggest that immigrant women from non-Western ethnic backgrounds in particular do not benefit from folic acid campaigns.[54,55] However, it is largely unknown which factors explain this ethnic discrepancy. In this context, one factor that particularly requires further research is language proficiency, being the logical prerequisite for the uptake of health information conveyed in such campaigns.

Selected maternal nutrients: the n-3 and n-6 fatty acids

The LC-PUFAs of the n-3 and n-6 essential fatty acid families (**Figure 1.1**) are key components of virtually all cell membranes. In addition, some of them are precursors of what are known as eicosanoids, hormone-like substances involved in a range of biological processes including those essential in pregnancy, like placental blood flow, cervix-ripening, and initiation of parturition.[56-59] The interest in maternal fatty acid status in relation to pregnancy initially stemmed from the apparent biochemical essential fatty acid shortage observed in healthy newborns[60] and from epidemiologic observations of longer gestations and higher birth weights in areas of high fish intake, such as the Faroe Islands.[61,62] Fish is the major source of the n-3 LC-PUFAs eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), and it was suggested that particularly these components of fish improved pregnancy outcomes. Subsequent observational and experimental studies, however, produced inconclusive results.[63-65]

Most observational as well as experimental studies relating maternal n-3 fatty acid intake to birth weight referred to intake in mid- or late pregnancy and failed to take into account the background n-6 fatty acid status. However, the metabolic pathways of the n-3 and n-6 fatty acids are highly interrelated,[58,59] and maternal n-6 fatty acids may interfere with the incorporation of n-3 fatty acids in plasma phospholipids.[66] A few studies have investigated the birth weight influences of both n-6 and n-3 fatty acids as measured in plasma phospholipids, though also with inconclusive results.[67-69]

Since the maternal essential polyunsaturated fatty acid status largely depends on maternal intake of these fatty acids, differences in dietary habits between ethnic groups may be relevant for pregnancy outcomes. A comparison between four European countries (the Netherlands, England, Finland, and Hungary) and one South American country (Ecuador)[70] revealed an interesting disparity: the Hungarian mothers showed the lowest n-3 fatty acid and highest n-6 fatty acid concentrations, while the Finnish mothers showed the opposite, assumingly reflecting the differences in dietary habits between Eastern and Western Europe. At the same time, the study showed the potential relevance of genetically determined metabolic differences:

compared to European women (of Caucasian ancestry), Ecuadorian women (of American-Indian ancestry) showed a significantly higher ratio between 20:3n-6 and its derivative arachidonic acid (AA, 20:4n-6) (i.e., 20:3n-6/20:4n-6), which is indicative of a lower delta-5 desaturase activity (see Figure 1.1.).[70] So far, no study has investigated within-country ethnic differences in the maternal n-3 and n-6 essential polyunsaturated fatty acid status, or to what extent these differences are related to intake (vs. metabolic) variation.

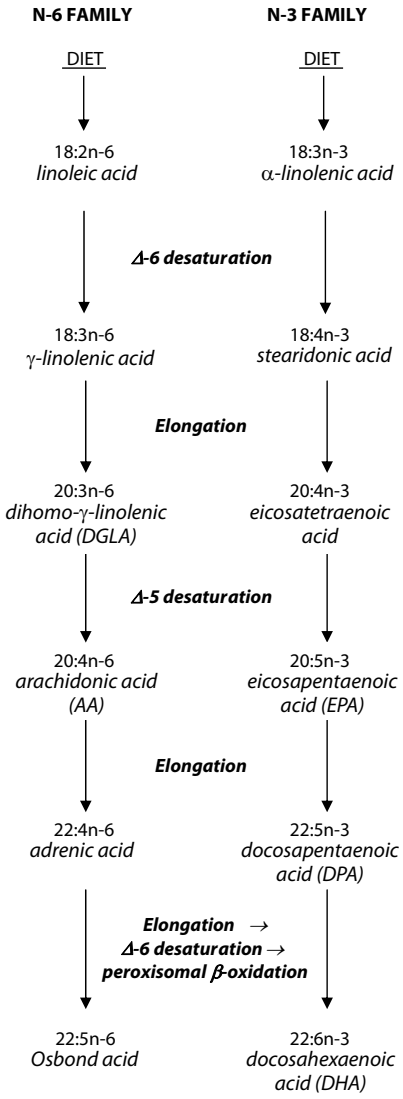

Figure 1.1 Schematic presentation of the n-3 and n-6 essential fatty acid families and their preferred metabolic pathway.

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Research questions

The following research questions will be addressed in the remaining chapters of this thesis:

Validity of nutrient analyses:

1. Is a pragmatic approach to blood sampling suitable for valid measurement of nutrient status in a large-scale epidemiologic study? (Chapter 2)

Ethnicity and maternal nutrition:

2. (a) Does periconceptional use of folic acid supplements differ between women from ethnic minority groups and Dutch women; (b) are there ethnic-specific determinants that can explain ethnic differences in folic acid supplement use; and (c) how important is language proficiency as a determinant of use among women who were born in non-Dutch-speaking, non-Western countries? (Chapter 3)
3. (a) How do early pregnancy fatty acid concentrations among ethnic minority women compare to the early pregnancy fatty acid concentrations among Dutch women; and (b) to what extent can fish intake as a source of n-3 LC-PUFAs account for the ethnic variation in maternal n-3 and n-6 LC-PUFA concentrations? (Chapter 4)

Ethnicity and birth weight:

4. (a) How do the term birth weight distributions of ethnic minority women compare to the term birth weight distribution of Dutch women; and (b) to what extent can ethnic differences in birth weight be explained by conventional physiologic and environmental (but non-nutritional) risk factors? (Chapter 5)

Maternal nutrition and birth weight:

5. Is there a role for folate depletion in the association of short interpregnancy intervals with birth weight and SGA risk at term? (Chapter 6)
6. How does the maternal n-3 and n-6 fatty acid status relate to fetal growth as measured by infant birth weight and SGA risk at term? (Chapter 7)

The general discussion (Chapter 8) presents a summary of the findings and their interpretation, and discusses the implications for future research, perinatal care, and public health policy.

References

1. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. Lancet 1989;2:577–80.
2. Osmond C, Barker DJ, Winter PD, Fall CH, Simmonds SJ. Early growth and death from cardiovascular disease in women. BMJ 1993;307:1519–24.
3. Barker DJ. Mothers, babies and health in later life. 2nd ed. Edinburgh: Churchill Livingstone, 1998.
4. Gluckman P, Hanson M. The fetal matrix: evolution, development and disease. Cambridge: Cambridge University Press, 2005.
5. van den Oord EJ. Ethnic differences in birth weight: maternal effects emerge from an analysis involving mixed-race US couples. Ethn Dis 2006;16:706–11.
6. Sheeder J, Lezotte D, Stevens-Simon C. Maternal age and the size of white, black, hispanic, and mixed infants. J Pediatr Adolesc Gynecol 2006;19:385–9.
7. Chike-Obi U, David RJ, Coutinho R, Wu SY. Birth weight has increased over a generation. Am J Epidemiol 1996;144:563–9.
8. David RJ, Collins JW. Differing birth weight among infants of U.S.-born blacks, African-born blacks, and U.S.-born whites. N Eng J Med 1997;337:1209–14.
9. Goldenberg RL, Cliver SP, Mulvihill FX, et al. Medical, psychosocial, and behavioral risk factors do not explain the increased risk for low birth weight among black women. Am J Obstet Gynecol 1996;175:1317–24.
10. Shiono PH, Rauh VA, Park M, Lederman SA, Zuskar D. Ethnic differences in birthweight: the role of lifestyle and other factors. Am J Public Health 1997;87:787–93.
11. Wilcox M, Gardosi J, Mongelli M, Ray C, Johnson I. Birth weight from pregnancies dated by ultrasonography in a multicultural British population. BMJ 1993;307:588–91.
12. Perry IJ, Beevers DG, Whincup PH, Bareford D. Predictors of ratio of placental weight to fetal weight in multiethnic community. BMJ 1995;310:436–9.
13. Harding S, Rosato MG, Cruickshank JK. Lack of change in birthweights of infants by generational status among Indian, Pakistani, Bangladeshi, Black Carribean, and Black African mothers in a British cohort study. Int J Epidemiol 2004;33:1279–85.
14. Doornbos JP, Nordbeck HJ, van Enk AE, Muller AS, Treffers PE. Differential birthweights and the clinical relevance of birthweight standards in a multiethnic society. Int J Gynaecol Obstet 1991;34:319–24.
15. van der Wal MF, Uitenbroek DG, van Buuren S. Geboortegewicht van Amsterdamse kinderen naar etnische afkomst (Birth weights of Amsterdam born children according to ethnic origin). TSG tijdschrift voor gezondheidswetenschappen 2000;78:15–20 (in Dutch).
16. Drooger JC, Troe JW, Borsboom GJ, et al. Ethnic differences in prenatal growth and the association with maternal and fetal characteristics. Ultrasound Obstet Gynecol 2005;26:115–22.
17. Troe EJ, Raat H, Jaddoe VW, et al. Explaining differences in birthweight between ethnic populations. The Generation R study. BJOG 2007;114:1557–65.
18. Keen CL, Clegg MS, Hanna LA, et al. The plausibility of micronutrient deficiencies being a significant contributing factor to the occurrence of pregnancy complications. J Nutr 2003;133(suppl 2):1597S–605S.

19. Mamelle N, Cochet V, Claris O. Definition of fetal growth restriction according to constitutional growth potential. Biol Neonate 2001;80:277–85.
20. Eaton PM, Wahrton PA, Wharton BA. Nutrient intake of pregnant Asian women at Sorrento Maternity Hospital, Birmingham. Br J Nutr 1984;52:457–68.
21. Suitor CW, Gardner JD, Feldstein ML. Characteristics of diet among a culturally diverse group of low-income pregnant women. J Am Diet Assoc 1990;90:543–9.
22. Siega-Riz AM, Bodnar LM, Savitz DA. What are pregnant women eating? Nutrient and food group differences by race. Am J Obster Gynecol 2002;186:480–6.
23. Arab L, Carriquiry A, Steck-Scott S, Gaudet MM. Ethnic differences in the nutrient intake adequacy of premenopausal US women: results from the third national health examination survey. J Am Diet Assoc 2003;103:1008–14.
24. Rees G, Brooke Z, Doyle W, Costeloe K. The nutritional status of women in the first trimester of pregnancy attending an inner-city antenatal department in the UK. J R Soc Health 2005;125:232–8.
25. Rees GA, Doyle W, Srivasta A, Brooke ZM, Crawford MA, Costeloe KL. The nutrient intakes of mothers of low birth weight babies - a comparison of ethnic groups in East London, UK. Matern Child Nutr 2005;1:91–9.
26. Gurr MI. Fats. In: Garrow JS, James WP, Ralph A. Human nutrition and dietetics. 10th ed. Edinburgh: Churchill Livingstone, 2000:97–120.
27. Garza C, Rasmussen KM. Pregnancy and lactation. In: Garrow JS, James WP, Ralph A. Human nutrition and dietetics. 10th ed. Edinburgh: Churchill Livingstone, 2000:437–48.
28. Ramakrishnan U, Manjrekar R, Rivera J, Gonzáles-Cossío T, Martorell R. Micronutrients and pregnancy outcome: a review of the literature. Nutr Res 1999;19:103–59.
29. Ladipo OA. Nutrition in pregnancy: mineral and vitamin supplements. Am J Clin Nutr 2000;72(suppl 1):280S–90S.
30. Fall CH, Yajnik CS, Rao S, Davies AA, Brown N, Farrant HJ. Micronutrients and fetal growth. J Nutr 2003;133(suppl 2):1747S–56S.
31. Jackson AA, Robinson SM. Dietary guidelines for pregnancy: a review of current evidence. Public Health Nutr 2001;4:625–30.
32. Kind K, Moore VM, Davies MJ. Diet around conception and during pregnancy – effects on fetal and neonatal outcomes. Reprod Biomed Online 2006;12:532–41.
33. Godfrey KM, Barker DJ. Fetal programming and adult health. Public Health Nutr 2001;4:611–24.
34. Smith GC. First trimester origins of fetal growth impairment. Semin Perinat 2004;28:41–50.
35. van Zee W, Hylkema C, eds. Kerncijfers Amsterdam 2004 (Key figures Amsterdam 2004). Amsterdam: Stadsdrukkerij Amsterdam, 2004 (in Dutch).
36. Wild CP, Andersson C, O'Brien NM, Wilson L, Woods JA. A critical evaluation of the application of biomarkers in epidemiological studies on diet and health. Br J Nutr 2001;86(suppl 1):S37–53.
37. Hunter D. Biochemical indicators of dietary intake. In: Willett W. Nutritional epidemiology. 2nd ed. New York/Oxford: Oxford University Press, 1998:174–243.

38. Scholl TO, Johnson WG. Folic acid: influence on the outcome of pregnancy. Am J Clin Nutr 2000;71 (suppl 5):1295S–303S.
39. Tamura T, Picciano MF. Folate and human reproduction. Am J Clin Nutr 2006;83:993–1016.
40. Cornel MC, de Smit DJ, de Jong-van den Berg LT. Folic acid – the scientific debate as a base for public health policy. Reprod Toxicol 2005;20:411–5.
41. Green R, Miller JW. Folate deficiency beyond megaloblastic anemia: hyperhomocysteinemia and other manifestations of dysfunctional folate status. Semin Hematol 1999;36:47–64.
42. Tamura T, Goldenberg RL, Freeberg LE, Cliver SP, Cutter GR, Hoffman HJ. Maternal serum folate and zinc concentrations and their relationships to pregnancy outcome. Am J Clin Nutr 1992;56:365–70.
43. Scholl TO, Hediger ML, Schall JI, Khoo CS, Fischer RL. Dietary and serum folate: their influence on the outcome of pregnancy. Am J Clin Nutr 1996;63:520–5.
44. Vollset SE, Refsum H, Irgens LM, et al. Plasma total homocysteine, pregnancy complications, and adverse pregnancy outcomes: the Hordaland Homocysteine Study. Am J Clin Nutr 2000;71:962–8.
45. Murphy MM, Scott JM, Arija V, Molloy AM, Fernandez-Ballart JD. Maternal homocysteine before conception and throughout pregnancy predicts fetal homocysteine and birth weight. Clin Chem 2004;50:1406–12.
46. Lindblad B, Zaman S. Malik A, et al. Folate, vitamin B12, and homocysteine levels in South Asian women with growth-retarded fetuses. Acta Obstet Gynecol Scand 2005;84:1055–61.
47. Sram RJ, Binkova B, Lnenickova Z, Solansky I, Dejmek J. The impact of plasma folate levels of mothers and newborns on intrauterine growth retardation and birth weight. Mutat Res 2005;591:302–10.
48. Charles DH, Ness AR, Campbell D, Smith GD, Whitley E, Hall M. Folic acid supplements in pregnancy and birth outcome: re-analysis of a large randomised controlled trial and update of Cochrane review. Paediatr Perinat Epidemiol 2005;19:112–24.
49. Baumslag N, Edelstein T, Metz J. Reduction of incidence of prematurity by folic acid supplementation in pregnancy. Br Med J 1970;1:16–7.
50. Rao S, Yajnik CS, Kanade A, et al. Intake of micronutrient-rich foods in rural Indian mothers is associated with the size of their babies at birth: Pune Maternal Nutrition Study. J Nutr 2001;131:1217–24.
51. Smits LJ, Essed GG. Short interpregnancy intervals and unfavourable pregnancy outcome: role of folate depletion. Lancet 2001;358:2074–7.
52. Ray JG, Singh G, Burrows RF. Evidence for suboptimal use of periconceptional folic acid supplements globally. BJOG 2004;111:399–408.
53. de Walle HE, de Jong-van den Berg LT. Growing gap in folic acid intake with respect to level of education in the Netherlands. Community Genet 2007;10:93–6.
54. Howell SR, Barnett AG, Underwood MR. The use of pre-conceptional folic acid as an indicator of uptake of a health message amongst white and Bangladeshi women in Tower Hamlets, east London. Fam Pract 2001;18:300–3.
55. Bakker MK, Cornel MC, de Walle HE. Kennis over en gebruik van periconceptioneel foliumzuur onder allochtone en westerse vrouwen, na de publiekscampagne in 1995 (Awareness and periconceptional use of folic acid among non-western and western women in the Netherlands following the 1995 publicity campaign). Ned Tijdschr Geneeskd 2003;147:2426–30 (in Dutch).

56. Olsen SF. Consumption of marine n-3 fatty acids during pregnancy as a possible determinant of birth weight. A review of the current epidemiologic evidence. Epidemiol Rev 1993;15:399–413.
57. Uauy R, Calderon F, Mena P. Essential fatty acids in somatic growth and brain development. World Rev Nutr Diet 2001;89:134–60.
58. Allen KG, Harris MA. The role of n-3 fatty acids in gestation and parturition. Exp Biol Med 2001;226:498–506.
59. Innis SM. Perinatal biochemistry and physiology of long-chain polyunsaturated fatty acids. J Pediatr 2003;143(suppl 4):S1–8.
60. Hornstra G, van Houwelingen AC, Simonis M, Gerrard JM. Fatty acid composition of umbilical arteries and veins: possible implications for the fetal EFA-status. Lipids 1989;24:511–7.
61. Olsen SF, Joensen HD. High liveborn birth weights in the Faroes: a comparison between birth weights in the Faroes and in Denmark. J Epidemiol Community Health 1985;39:27–32.
62. Olsen SF, Hansen HS, Sorensen T, et al. Intake of marine fat, rich in (n-3) polyunsaturated fatty acids, may increase birthweight by prolonging gestation. Lancet 1986;2:367–9.
63. Jensen CL. Effects of n-3 fatty acids during pregnancy and lactation. Am J Clin Nutr 2006;83(suppl 6):1452S–7S.
64. 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;83:1337–44.
65. Makrides M, Duley L, Olsen SF. Marine oil, and other prostaglandin precursor, supplementation for pregnancy uncomplicated by pre-eclampsia or intrauterine growth restriction. Cochrane Database Syst Rev 2006;3:CD003402.pub2.
66. Gronn M, Gorbitz C, Christensen E, et al. Dietary n-6 fatty acids inhibit the incorporation of dietary n-3 fatty acids in thrombocyte and serum phospholipids in humans: a controlled dietetic study. Scand J Clin Lab Invest 1991;51:255–63.
67. Grandjean P, Bjerve KS, Weihe P, Steuerwald U. Birthweight in a fishing community: significance of essential fatty acids and marine food contaminants. Int J Epidemiol 2001;30:1272–8.
68. Rump P, Mensink RP, Kester AD, Hornstra G. Essential fatty acid composition of plasma phospholipids and birth weight: a study in term neonates. Am J Clin Nutr 2001;73:797–806.
69. Elias SL, Innis SM. Infant plasma trans, n-6 and n-3 fatty acids and conjugated linoleic acids are related to maternal plasma fatty acids, length of gestation and birth weight and length. Am J Clin Nutr 2001;73:807–14.
70. Otto SJ, van Houwelingen AC, Antal M, et al. Maternal and neonatal essential fatty acid status in phospholipids: an international comparative study. Eur J Clin Nutr 1997;51:232–42.

Chapter 2

Can whole-blood samples be stored over 24 hours without compromising stability of C-reactive protein, retinol, ferritin, folate, and fatty acids in epidemiologic research?

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Technical brief

Ideally, blood samples for biomarker measurement are collected centrally and processed immediately to avoid any unwanted changes in concentrations that could affect validity. In large-scale epidemiologic and clinical studies, however, this theoretical goal must give way to a more pragmatic approach.[1,2] The Amsterdam Born Children and their Development (ABCD) study is a clinically based cohort study of pregnant women in Amsterdam (the Netherlands) in which we use, for practical and ethical reasons, blood collection in conjunction with existing schemes of care, with samples subsequently sent to a central laboratory by mail or courier. As a consequence, delay times between collection and processing may exceed 24 h, to an incidental maximum of 96 h. Although the stabilities of the biomarkers of interest, i.e., C-reactive protein (CRP), retinol, ferritin, folate, and fatty acids (FAs) have been studied previously,[1-9] the variety of designs (e.g., storage ≤24 h) hampers the applicability of existing results to our research and similar studies. We therefore investigated the appropriateness of our standardized, practice-based approach of blood collection by assessing stability in samples stored up to 96 h (the maximum delay time possible when samples are sent by mail) with a focus on the first 28 h (the maximum delay time allowed in the ABCD study).

Blood samples were collected from 41 generally healthy female volunteers, 22–56 years of age. One woman was at that time 16 weeks pregnant, but because her measurements did not deviate, we decided not to exclude her from analysis. Written informed consent was obtained from all participants. For 20 women, 28 mL of blood was collected in seven 4-mL Vacuettes (Greiner BV) for the preparation of serum. For the other 21 women, 28 mL blood was collected in seven 4-mL Vacutainer EDTA tubes (Becton and Dickinson BV) for the preparation of plasma. One tube from each woman was centrifuged (1600*g* for 10 min) between 1 and 2 h after blood collection ($t_{0;}$ baseline), and the remaining tubes were stored in a cabinet at room temperature (~21° C) and centrifuged 2 (t_2), 4 (t_4) , 24 (t_{24}), 26 (t_{26}), 28 (t_{28}), or 96 (t_{96}) h after baseline. Time points were chosen to mimic courier and postal conditions seen in the ABCD study, 4 h being the anticipated maximum delay time when blood is sent by courier, 28 h being the anticipated maximum delay time when blood is sent by overnight-mail, and 96 h being the anticipated maximum delay time when blood is sent by mail but over the weekend.

Aliquots (1 mL) of plasma and serum were stored at −80°C and transferred on dry ice to collaborating laboratories for analysis within 1 week. In serum, CRP concentrations were measured by use of the Dade-Behring high-sensitivity assay.[10] Ferritin and folate concentrations were measured by immunoassay with chemiluminescence detection on the Advia Centaur System (Bayer Group),[11] and retinol was measured with an isocratic reversed-phase HPLC method (Waters Ltd.).[12] Intra- and interassay CVs, as determined previously, were 4.1% and 5.2%, respectively, for CRP; 2.5% and 5.4% for ferritin; 7.9% and 6.1% for folate; and 3.3% and 7.3% for retinol. The FA composition of plasma phospholipids was determined by capillary gas chromatography with flame ionization detection (HP5890 series II, Hewlett

Packard) as described by Al et al.[13] Absolute (mg/L of plasma) and relative (percentage of total phospholipid-associated FAs) amounts were calculated for the major saturated FAs and the major mono- and polyunsaturated FAs of the n-3, n-6, and n-9 families, each constituting ≥0.1% of total FAs. CVs varied from 1.3% for arachidonic acid (20:4n-6) to 5.6% for Mead acid (20:3n-9). For FA analysis, all paired samples were assayed in the same run.

After verification of the homogeneity of variance and identification of outliers [two measurements of folate processed at t_{26}, one baseline measurement of linoleic acid (18:2n-6), one baseline measurement of Mead acid (20:3n-9) and one measurement of adrenic acid (22:4n-6) at t_{24}, all excluded from analysis], we performed repeated-measures ANOVA to explore time effects over 96 h and to calculate the mean percentage change per time point. Because we assumed that the stability depended on both reliability and validity (because true changes over time can be established only if measurement error is small), we assessed stability at 28 h by calculating intraclass correlation coefficients[5] (two-way mixed-effect model with single-measure reliability; SPSS), and by computing Spearman rank correlations for a (n = 50) bootstrap sample. In the latter procedure, we took for all women random samples from all values and calculated correlation coefficients with baseline values. Because these correlations depend on both the distribution of the individual values and the stability, this measure provides a nonparametric estimate of the "noise" to be expected because of unknown variance of delay, in particular in regression-like analyses. Additional analyses of within- and between-subject CVs, assessing the magnitude of the variation attributable to time in relation to the variation between individuals,[1] can be viewed in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol51/issue1/.

The changes in concentration, reliability coefficients, and Spearman rank correlations for measured analytes are shown in **Table 2.1**. CRP was excluded from the reliability and validity analyses because we considered the number of observations above the clinically significant detection level (0.5 mg/L) insufficient (insufficient power). A graphic representation of changes across all time points is presented in the online Data Supplement. For CRP, retinol, and ferritin, changes in concentration during the 96-h storage period were small and not significant (≤10%, $P \geq 0.1$) For folate, the mean concentration changed significantly over time ($P = 0.037$), with the sharpest decrease (8.7%) in the first 2 h of storage. Reliability and validity measures were high for all serum analytes (intraclass correlation coefficient ≥ 0.96; Spearman rank correlation coefficient ≥ 0.80).

Significant changes over time were observed for most FAs, but only for linoleic acid and adrenic acid were changes ≥10%. The linoleic acid concentration decreased 14% over the 96-h storage period ($P \leq 0.001$), and the concentration of adrenic acid increased 16% ($P \leq 0.001$). For the majority of FAs, reliability coefficients were ≥0.97. For Mead acid, the reliability coefficient was lower (0.81). Spearman rank correlations were ≥0.93 for all FAs except Mead acid (0.78).

Our results demonstrate that a pragmatic approach for data collection does not affect

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the stability of measurements of CRP, retinol, ferritin, and most FAs, because these markers showed limited variance within a 96-h storage period. For folate, linoleic acid, and adrenic acid, significant changes (≥10%) over time were observed, but these did not significantly affect measures of 28-h validity.

Although previous studies on the stability of CRP and ferritin showed results similar to ours,[3-6,8] studies on retinol are conflicting. Both Key et al.[1] and Hankinson et al.[2] have reported small decreases (≤5.0%), whereas Mejia et al.[7] observed no change in concentration. We also observed no significant changes, possibly as result of the storage of our samples in the dark to protect vitamin A from photodestruction.[14]

Folate is known for its sensitivity for both heat and light, and substantial decreases (>10%) during storage have been reported.[8,9] Zhang et al.,[9] who found a 15% decrease after 24 h of storage, concluded that this is within acceptable limits for clinical analysis. Similarly, we observed no relevant time-related changes in the 28-h stability analyses.

To our knowledge, no previous studies have reported on the stability of FAs in plasma phospholipid fraction. Generally, we observed only small changes in concentrations, which did not significantly affect coefficients of correlation. For Mead acid, however, reliability and validity coefficients were more influenced by variability in concentration, possibly because of the small magnitude of the individual values (<1%).[5] Surprisingly, we observed not only decreases in FA concentrations (assumingly attributable to FA peroxidation), but also increases. A second process may be ongoing during storage: the formation of erythrocyte microparticles. In vitro, glycolysis will lower the glucose concentration in erythrocytes, causing depletion of ATP. Consequently, the erythrocytes cannot maintain membrane integrity, and microparticles will be released.[15] Over time, the concentration of plasma phospholipid-associated FAs may thus become enriched with erythrocyte membrane phospholipids containing relatively high amounts of polyunsaturated FAs.

Our study has some limitations. One limitation is that we did not measure in duplicate to adjust for intraassay variation. In addition, serum samples were not analyzed within one run to minimize interassay variability. However, standardized procedures were used for analysis, with CVs ≤ 7.9% for serum analytes and ≤ 5.6% for plasma phospholipid-associated FAs. In addition, the reliability analyses showed high agreement between follow-up and baseline values. Another limitation is that samples were not randomized before analysis. Possible bias from order of draw, although unlikely, can therefore not be ruled out. A third limitation is that we were not able to test the reliability and validity of CRP over the 28-h storage period. However, changes in concentration were small (≤5.5%), which, as mentioned before, is in line with results from other studies.[3,5]

In conclusion, this study shows that in the context of epidemiologic studies investigating (nutritional) status during routine care, a pragmatic approach to blood collection may validly be applied to determine CRP, retinol, ferritin, folate, or FA status. Although storage will diminish the precision of estimates, standard (correlational) epidemiologic analyses will not be compromised in samples stored for a maximum of 28 h.

Table 2.1 Stability of serum analytes and fatty acids in plasma phospholipids: changes in concentration after storage for 2 to 96 h, and 28-h reliability and validity measures

	Mean (SE) baseline value	Change from baseline, %						*P*, time effect[a]	28-h reliability (ICC)[b]	28-h validity (Spearman)[c]
		+2h	+4h	+24h	+26h	+28h	+96h			
Serum analytes										
CRP,[d] mg/L (n = 6)	18.4 (14.2)	−3.0	−4.4	−5.5	−5.2	−2.9	−3.0	0.370	-	-
CRP,[e] mg/L (n = 5)	4.2 (0.9)	−10.0	−1.4	−6.2	−3.8	1.4	2.8	0.105	-	-
Retinol, µmol/L	3.01 (0.2)	2.7	0.8	1.7	0.4	1.8	1.1	0.311	.97	.96
Ferritin, µg/L	45 (11)	−0.4	−1.4	0.5	2.1	3.1	2.6	0.110	.99	.99
Folate, nmol/L	19.7 (2.9)	−8.7	−10.8	−13.6	−18.7	−11.5	−16.5	0.037	.97	.87
Fatty acids in plasma phospholipids, % by weight of total fatty acids										
16:0	29.16 (0.40)	−0.5	−0.2	0.7	0.7	0.6	2.4	<0.001	.98	.93
18:0	12.41 (0.27)	−0.4	0.1	2.0	2.0	1.7	6.0	<0.001	.98	.96
18:1n-9	8.39 (0.24)	−0.8	−1.0	−1.9	−1.7	−2.2	−3.8	<0.001	.97	.96
20:3n-9	0.38 (0.02)	6.1	7.1	2.3	0.1	0.5	4.9	0.256	.81	.78
18:2n-6	20.97 (0.59)	−0.4	−1.1	−4.7	−4.9	−5.3	−14.0	<0.001	.99	.97
20:3n-6	3.00 (0.14)	−0.4	−0.3	1.0	1.1	1.5	4.0	<0.001	.99	.98
20:4n-6	8.73 (0.30)	−0.2	−0.3	−0.1	0.0	0.2	1.4	0.021	.99	.98
22:4n-6	0.30 (0.01)	−0.2	0.9	3.1	4.1	4.8	16.2	<0.001	.99	.99
22:5n-6	0.21 (0.02)	0.2	−0.2	0.5	1.7	2.7	6.8	<0.001	.99	.97
18:3n-3	0.25 (0.01)	−0.9	−1.7	−4.7	−3.5	−4.9	−9.7	<0.001	.97	.95
20:5n-3	1.03 (0.17)	−0.2	−1.0	−1.9	−2.0	−1.7	−5.2	0.004	.99	.99
22:5n-3	0.78 (0.04)	0.5	0.9	3.0	3.6	4.3	9.6	<0.001	.99	.98
22:6n-3	3.56 (0.22)	0.0	0.1	1.7	2.0	2.6	5.8	<0.001	.99	.98

[a] *Huynh-Feldt correction in repeated measures ANOVA (accounting for unequal variances of differences across groups).*

[b] *Mean Spearman rank correlation for bootstrap sample of n = 50.*

[c] *ICC, intraclass correlation coefficient.*

[d] *Only six individuals with values above detection level (0.5 mg/L), power too low for reliability and validity measurements.*

[e] *One of the six individuals had extreme high values (baseline value, 89 mg/L), analyses repeated with five individuals.*

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Acknowledgements

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Data Supplement

Table S2.1 Variability of serum analytes and phospholipid-associated fatty acids in samples stored up to 28 h

	CV(%)	
	Within	Between
Serumanalytes		
CRP,[a] mg/L (n = 6)	-	-
CRP,[b] mg/L (n = 5)	-	-
Retinol, µmol/L	0.9	22.5
Ferritin, µg/L	1.6	102.3
Folate, nmol/L	7.7	61.8
Fatty acids in plasma phospholipids, % by weight of total fatty acids		
16:0	0.5	5.7
18:0	1.2	9.7
18:1n-9	1.0	11.8
20:3n-9	3.2	23.1
18:2n-6	2.4	12.2
20:3n-6	1.2	20.7
20:4n-6	0.1	15.4
22:4n-6	2.4	21.8
22:5n-6	1.4	39.1
18:3n-3	2.4	25.1
20:5n-3	1.0	72.1
22:5n-3	1.5	23.0
22:6n-3	1.2	26.6

[a] Only six individuals with values above detection level (0.5 mg/L), power too low for reliability and validity measurements.

[b] One of the six individuals had extreme high values (baseline value, 89 mg/L), analyses repeated with five individuals.

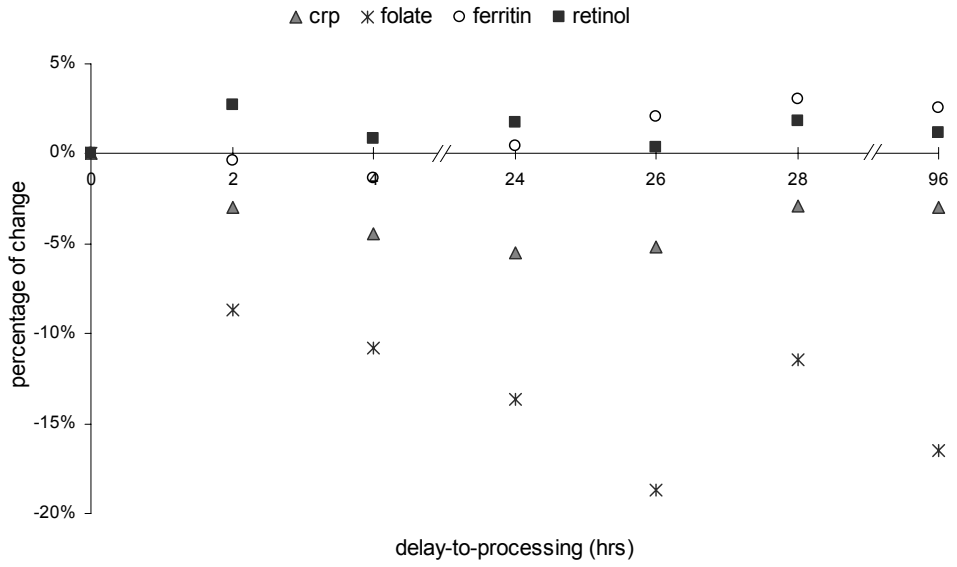

Figure S2.1 Mean percentage of change in concentration of serum analytes during storage of whole blood: C-reactive protein (n = 6), retinol (n = 20), ferritin (n = 20), and folate (n = 20).

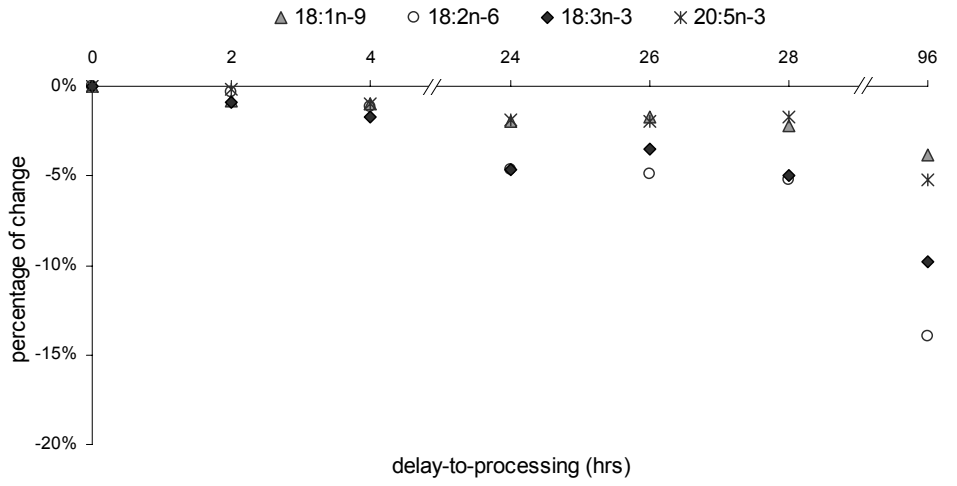

Figure S2.2 Mean percentage of change in concentration of plasma fatty acids in phospholipid fraction during storage of whole blood (n = 21): fatty acids showing a decrease in concentration.

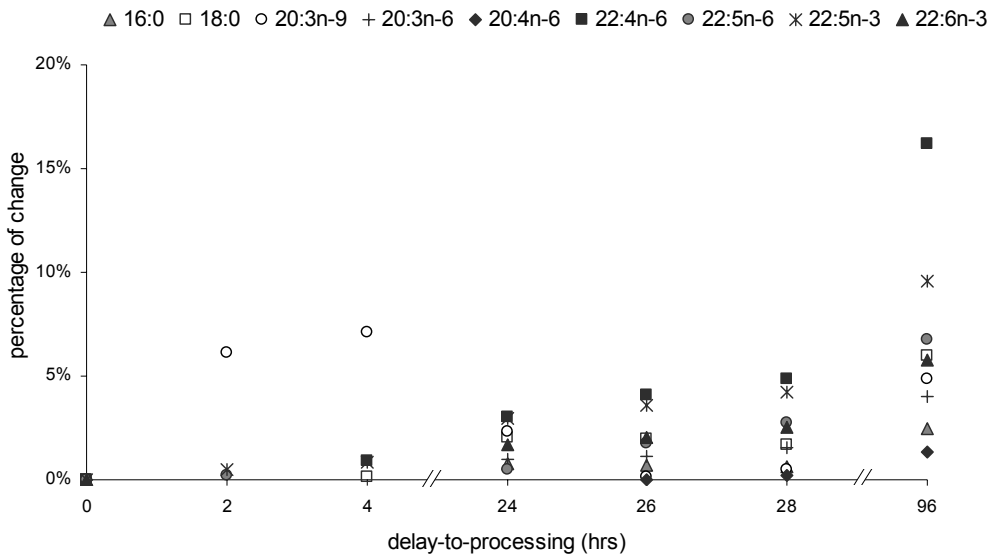

Figure S2.3 Mean percentage of change in concentration of plasma fatty acids in phospholipid fraction during storage of whole blood (n = 21): fatty acids showing an increase in concentration.

References

1. Key T, Oakes S, Davey G, et al. Stability of vitamins A, C, and E, carotenoids, lipids, and testosterone in whole blood stored at 4 degrees C for 6 and 24 hours before separation of serum and plasma. Cancer Epidemiol Biomarkers Prev 1996;5:811–4.
2. Hankinson SE, London SJ, Chute CG, et al. Effect of transport conditions on the stability of biochemical markers in blood. Clin Chem 1989;35:2313–6.
3. Aziz N, Fahey JL, Detels R, Butch AW. Analytical performance of a highly sensitive C-reactive protein-based immunoassay and the effects of laboratory variables on levels of protein in blood. Clin Diagn Lab Immunol 2003;10:652–7.
4. Birgegard G. Serum ferritin: physiological and methodological studies. Clin Chim Acta 1980;103:277–85.
5. Giltay EJ, Geleijnse JM, Schouten EG, Katan MB, Kromhout D. High stability of markers of cardiovascular risk in blood samples. Clin Chem 2003;49:652–5.
6. Kubasik NP, Ricotta M, Hunter T, Sine HE. Effect of duration and temperature of storage on serum analyte stability: examination of 14 selected radioimmunoassay procedures. Clin Chem 1982;28:164–5.
7. Mejia LA, Arroyave G. Determination of vitamin A in blood. Some practical considerations on the time of collection of the specimens and the stability of the vitamin. Am J Clin Nutr 1983;37:147–51.
8. Chu SY, MacLeod J. Effect of three-day clot contact on results of common biochemical tests with serum. Clin Chem 1986;32:2100.
9. Zhang DJ, Elswick RK, Miller WG, Bailey JL. Effect of serum-clot contact time on clinical chemistry laboratory results. Clin Chem 1998;44:1325–33.
10. Ledue TB, Weiner DL, Sipe JD, Poulin SE, Collins MF, Rifai N. Analytical evaluation of particle-enhanced immunonephelometric assays for C-reactive protein, serum amyloid A and mannose-binding protein in human serum. Ann Clin Biochem 1998;35:745–53.
11. Owen WE, Roberts WL. Comparison of five automated serum and whole blood folate assays. Am J Clin Pathol 2003;120:121–6.
12. Talwar D, Ha TK, Cooney J, Brownlee C, O'Reilly DS. A routine method for the simultaneous measurement of retinol, alpha-tocopherol and five carotenoids in human plasma by reverse phase HPLC. Clin Chim Acta 1998;270:85–100.
13. Al MD, van Houwelingen AC, Kester AD, Hasaart TH, de Jong AE, Hornstra G. Maternal essential fatty acid patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status. Br J Nutr 1995;74:55–68.
14. Barreto-Lins MH, Campos FA, Azevedo MC, Flores H. A re-examination of the stability of retinol in blood and serum, and effects of a standardized meal. Clin Chem 1988;34:2308–10.
15. Lutz HU, Liu SC, Palek J. Release of spectrin-free vesicles from human erythrocytes during ATP depletion. I. Characterization of spectrin-free vesicles. J Cell Biol 1977;73:548–60.

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Chapter 3

Folic acid knowledge and use in a multi-ethnic pregnancy cohort: the role of language proficiency

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Abstract

Objective

To investigate the role of language proficiency as determinant of folic acid knowledge and use in a multi-ethnic pregnancy cohort.

Design

Prospective cohort study.

Setting and population

Pregnant women from Amsterdam attending obstetric care for their first antenatal visit. Number approached: 12 373 women, response rate: 67% (8266 women aged 14–49 years). Ethnicity was based on the country of birth: the Netherlands, Surinam, Antilles, Turkey, Morocco, Ghana, other non-Western, and other Western countries.

Main outcome measures

Knowledge about and use of folic acid supplements in pregnancy as elicited in a multilingual questionnaire, as well as determinants of these in ethnic groups separately.

Results

Both periconceptional folic acid use and knowledge were significantly lower among Ghanaian, Moroccan, Turkish, and other non-Western women than among women born in the Netherlands or other Western countries. Language proficiency in Dutch was a major determinant of knowledge in all ethnic groups with a mother tongue other than Dutch [adjusted odds ratios (OR): Western 3.2, non-Western (all countries combined) 7.5], while educational attainment was of secondary importance. Knowledge in turn was the strongest determinant of use (adjusted OR: Western 17.4, non-Western 27.0).

Conclusions

Periconceptional folic acid supplement use among women born in non-Dutch-speaking non-Western countries is low, reflecting a lack of knowledge that is determined by the inability to speak and understand the language of the country of residence. Measures to tackle this problem include the provision of linguistically appropriate information via ethnic health advisors, and language courses integrating health education for immigrants.

Introduction

The benefits of prenatal care for a healthy pregnancy and subsequent birth outcome are generally acknowledged.[1,2] Inadequate prenatal care has been associated with an increase in adverse events, including perinatal morbidity[3] as well as perinatal and neonatal mortality.[4,5] Despite this awareness and political efforts to reduce inequalities in health care in many Western countries,[6,7] inadequate prenatal care such as a late initiation of care and a lower uptake of screening is still observed, especially in ethnic minority groups.[2,8,9]

Racial and ethnic disparities in health care utilisation, in general, have been related to numerous factors. These include the cultural competence of care providers, i.e., the extent to which they are aware of "foreign" cultural health beliefs, as well as patients' social class, educational level, insurance provision, level of acculturation, and language proficiency.[10-13] The latter aspect – the ability to speak and understand the language of the country of residence – may be crucial, as it affects one's health care utilisation in different ways. Being sufficiently fluent enables one (1) to find his or her way within the health care system; (2) to obtain and understand (written) information on health matters, directly and indirectly (e.g., advertisements, product information); and (3) to communicate with the health care provider (and vice versa).[14,15]

An important aspect of prenatal care is the use of folic acid supplements, as supplementation is an effective and proven measure to prevents birth defects.[16] In various European countries, publicity campaigns to increase awareness about the vitamin have been implemented since the 1990s.[16] Although the campaigns were aimed at all women in the reproductive age, studies suggest that particularly women from ethnic minority groups did not benefit.[17,18] The present study aimed to identify the role of language proficiency, among other factors, as determinant of folic acid knowledge and use in a multi-ethnic pregnancy cohort in the Netherlands.

Methods

We used data from the Amsterdam Born Children and their Development (ABCD) study. The ABCD study is a prospective cohort study, aimed at examining the relationship between maternal lifestyle and psychosocial conditions during pregnancy and the child's health at birth as well as in later life. Between January 2003 and March 2004, pregnant women living in Amsterdam were invited to enrol in the ABCD study at their first antenatal visit to participating obstetric care providers (general practitioners, midwives and hospital gynaecologists; overall participation rate 96%). All approached women (12 373) were registered by way of a form covering personal data such as name, address, date of birth, and country of birth. After 2 weeks, a questionnaire, covering sociodemographic data, obstetric history, lifestyle, dietary habits, and psychosocial factors, was sent to the pregnant woman's home address as given

on above-mentioned registration form with the request to return it by prepaid mail. The questionnaires were in Dutch, but were accompanied by an English, Turkish, or Arabic translation depending on the woman's country of birth. Turkish- and Arabic-speaking women with reading difficulties or illiteracy were invited to contact one of a group dedicated trained female interviewers for oral administration. With these supportive measures, the study group enhanced participation of all women, regardless of Dutch language proficiency, educational level, or ethnic origin. Questionnaires were returned by 8266 women (response rate 67%). Approval for the study was obtained from the Medical Ethical Committees of participating hospitals and the Registration Committee of Amsterdam.

In the questionnaire, the knowledge about and use of folic acid supplements were assessed by two questions: "Have you ever read or heard about the use of folic acid by women planning to become pregnant?" and "Have you taken folic acid, either as a single supplement or as part of a multivitamin supplement, before or during your pregnancy?".[19] Women who had not taken supplements were additionally asked to select their main reason for nonuse: (1) I do not know anything about folic acid, (2) I did not know anything about folic acid until I got pregnant, (3) I object against using anything during my pregnancy, (4) I was pregnant sooner than expected, and (5) other reason(s).

Ethnicity was based on the woman's self reported country of birth. Subclassification was based on native language and cultural background, separating the Dutch-speaking countries [the Netherlands, Surinam, and the Netherlands Antilles (including Aruba)] from the countries with a mother tongue other than Dutch, and separating within the latter group the non-Western countries (Turkey, Morocco, Ghana, and other non-Western countries) from the Western countries (not specified).[20] Language proficiency was addressed by questioning how well one could understand Dutch, with responses classified into three categories: (1) does not speak Dutch at all or only with great difficulty (low proficiency), (2) speaks Dutch with some difficulty (intermediate proficiency), (3) speaks Dutch without difficulty (high proficiency). Information on other possible determinants, previously associated with folic acid knowledge or use,[21-24] included pregnancy intention, measured by questioning whether the respondent had wanted to become pregnant (yes/ no); previous pregnancy experience (parity 0/ parity ≥1); educational attainment (years of education after primary school: 0–5/ 6–10/ ≥11 years); and age (≤24/ 25–29/ 30–34/ ≥35 years).

Statistical analyses were conducted using SPSS version 11.5.2 for Windows in a subgroup of 8050 women for whom data on determinant and outcome variables were complete. We first compared all ethnic groups with respect to their language proficiency, folic acid knowledge, and folic acid use, using chi-square tests. We subsequently determined the independent explanatory factors for folic acid knowledge using forward stepwise regression analyses for the combined Western and non-Western ethnic groups (P for inclusion: <0.05, P for exclusion: >0.10). Potential factors were age, education, previous pregnancy experience, pregnancy intention, and proficiency in Dutch. All analyses were repeated to determine the independent

factors explaining folic supplement use. In these analyses, folic acid knowledge was included as potential determinant, as we expected this a prerequisite for folic acid use. Adjusted odds ratios (OR) are reported as the index of association.

Results

Between ethnic groups, significant differences existed in Dutch language proficiency, folic acid knowledge, and folic acid use (**Table 3.1**). While 58% of the women born in Western countries were able to speak and understand Dutch, less than half of the women born in non-Western countries were (Turkey 32%, Morocco 44%, Ghana 26%, and other non-Western countries 34%, $P < 0.001$). Knowledge about periconceptional use of folic acid was most prevalent among women born in the Netherlands (95%) and other Western countries (86%) and least prevalent among women born in non-Dutch, non-Western countries (Ghana 28%, Turkey 34%, Morocco 40%, and other non-Western countries 48%, $P < 0.001$). Similarly, periconceptional use of folic acid supplements was highest among women born in the Netherlands (86%) and other Western countries (78%) and lowest among women born in Ghana (21%), Morocco (24%), Turkey (25%), and other non-Western countries (41%) ($P < 0.001$). For all foreign-born women, both Western and non-Western, the main reason given for not taking folic acid was not having (prior) knowledge about this vitamin. For women born in the Netherlands, the main reasons were the timing of pregnancy (pregnant sooner than expected) and the absence of (prior) knowledge.

For all ethnicities with a mother tongue other than Dutch, knowledge of folic acid was strongly associated with language proficiency (**Table 3.2**). In the Western ethnic group, women with a high proficiency in Dutch were 3.2 times more likely [95% confidence interval (CI) 1.9–5.3] to have knowledge about folic acid than women with a low proficiency. In the non-Western ethnic group, women with a high proficiency in Dutch were even 7.5 times more likely (95% CI 5.8–9.7) to have knowledge about folic acid than women with a low proficiency. The second important factor determining folic acid knowledge in the non-Western group was educational attainment (high versus low educational level: OR 5.2, 95% CI 3.6–7.5). Exploration of country-specific determinants (see supplementary tables) showed that the main result – proficiency in Dutch as primary determinant – applied for all countries but one. For Ghanaian women, educational level was a more prominent explaining factor, with a secondary role for language proficiency (Table S3.1).

Knowledge of folic acid was by far the strongest determinant of folic acid supplement use in all the ethnic groups (**Table 3.2** and Table S3.2). In the Western group, women who had heard or read about folic acid were 17.4 (95% CI 10.1–29.8) times more likely to have taken folic acid supplements than women who had no folic acid knowledge. In the non-Western group this ratio was 27.0 (95% CI 19.9–36.7). In addition, women in both groups

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Table 3.1 Language proficiency, folic acid knowledge, folic acid use, and reasons for nonuse*

	Dutch-speaking			**Non-Dutch-speaking**				
	Netherlands (n = 5038)	Surinam (n = 438)	Netherlands Antilles (n = 93)	Turkey (n = 325)	Morocco (n = 558)	Ghana (n = 187)	Other non-Western (n = 694)	Other Western (n = 717)
Proficiency in Dutch (%)								
Low	0	1	1	43	33	45	38	23
Intermediate	0	1	8	25	23	29	27	19
High	100	98	91	32	44	26	34	58
Folic acid knowledge (%)								
No	5	25	29	66	60	72	52	14
Yes	95	75	71	34	40	28	48	86
Folic acid use (%)								
No	14	49	40	75	76	79	59	22
Yes	86	51	60	25	24	21	41	78
Main reason for not using folic acid (%)								
Do not know anything about folic acid	16	36	46	72	58	81	71	38
Did not know anything about folic acid until pregnancy	21	18	27	8	8	5	12	18
Object against using anything during pregnancy	9	12	12	9	14	3	5	8
Was pregnant sooner than expected	33	19	6	6	9	6	7	18
Other	21	16	9	5	11	5	6	18

** Figures may not add up to 100% exactly because of rounding up errors.*

delivering their first child were more likely to take folic acid supplements than primi- and multiparous women. All influence of Dutch proficiency was mediated by knowledge as we found no associations between language proficiency and folic acid use in the country-specific analyses (Table S3.2) and only a very weak remnant effect in the combined analysis for the non-Western group.

Discussion

From this unselected community-based multi-ethnic cohort study, three important findings emerge. First, the use of folic acid supplements is disturbingly low among pregnant non-Western women. Second, the use of folic acid is determined primarily by knowledge about folic acid. Finally, the key factor for knowledge about folic acid in both Western and non-Western ethnic groups is language proficiency. These results imply that inadequate use of folic acid supplements among women born in non-Western countries is the result of a language-mediated knowledge gap.

In agreement with results from previous smaller studies in the Netherlands and the UK,[17,18] we observed the lowest use of folic acid supplements among women born in non-Western countries. Cultural and religious beliefs have been associated with an inclination to use traditional (herbal) remedies and a reluctance to use prescribed medicines or follow medical advice,[25,26] which may explain lower usage of folic acid among foreign-born pregnant women. However, this explanation seems unlikely in this population as, overall, only 9% of the foreign-born women indicated that her reason for nonuse was a reluctance to use anything during pregnancy. In contrast, more than 70% of these women indicated the lack of (prior) knowledge as her main reason for not having taken folic acid supplements.

Theoretically, a change towards a healthier behaviour – in this case folic acid supplement use – is preceded by awareness of the problem.[27] Our data supports this view: we found knowledge to be the major determinant of use in both non-Western and Western groups. Interestingly, in both groups, a previous pregnancy experience influenced folic acid use, but not knowledge, in an negative way. In general multiparous women may be less inclined to adopt healthy behaviour than nulliparous women if they can rely on successful past experiences, i.e., the delivery of a healthy child. Previous studies in different ethnic populations have indeed observed a lower attendance and uptake of prenatal care among multiparous women.[28,29]

Knowledge of periconceptional folic acid use, reflecting a woman's comprehension of health education, depended on two factors: proficiency in the dominant language and educational attainment. Logically, being unable to (fully) understand the language of the country of residence makes it difficult or even impossible for one to absorb health information conveyed in that language. A mass media campaign in the dominant language will therefore be ineffective in educating ethnic minority groups.[30] More is to be expected from integrating

Table 3.2 Determinants of folic acid knowledge and use in ethnic groups with a mother tongue different from Dutch: forward regression analysis

	Knowledge				**Use**			
	Western (n = 717)		Non-Western (n = 1764)		Western (n = 717)		Non-Western (n = 1764)	
Determinants	Percent with knowledge	OR*	Percent with knowledge	OR	Percent with use	OR	Percent with use	OR
Proficiency in Dutch								
Low	76	1	21	1	72		16	1
Intermediate	83	1.7 (0.9–3.0)	31	1.5 (1.2–2.0)	73		25	1.3 (0.9–1.9)
High	92	3.2 (1.9–5.3)	69	7.5 (5.8–9.7)	83		50	1.6 (1.2–2.2)
Age (years)								
≤24	67	0.7 (0.3–1.5)	31		47	0.4 (0.2–0.8)	21	0.8 (0.5–1.1)
25–29	79	1	42		73	1	29	1
30–34	90	2.4 (1.3–4.2)	50		83	1.3 (0.8–2.4)	41	1.8 (1.3–2.5)
≥35	91	2.4 (1.3–4.5)	39		83	1.6 (0.8–2.9)	31	1.4 (0.9–2.2)
Education (years)								
0–5	71	1	29	1	57	1	20	1
6–10	88	2.2 (1.2–3.8)	48	1.9 (1.5–2.4)	81	2.0 (1.1–3.5)	36	1.3 (1.0–1.8)
≥11	91	2.4 (1.3–4.5)	71	5.2 (3.6–7.5)	84	1.8 (1.0–3.4)	62	2.4 (1.6–3.7)
Pregnancy experience								
Parity ≥ 1	87		39		73	1	6	1
Parity = 0	86		43		81	2.1 (1.4–3.4)	36	2.1 (1.6–2.8)
Pregnancy intention								
No	76		28	1	66		20	
Yes	87		43	1.7 (1.2–2.5)	79		32	
Folic acid knowledge								
No					25	1	6	1
Yes					87	17.4 (10.1–29.8)	66	27.0 (19.9–36.7)

** OR and 95% CI; presented only for determinants that are significantly associated with the outcome variable, adjusted for the other significant determinants.*

information about pregnancy and prenatal care in naturalisation or language courses for immigrants, an approach which also takes into account the additional barrier of educational background. Generally, higher educated women benefit more from health education than lower educated women.[24] It is plausible that women who attained a higher educational level actively search for information, rather than passively wait for it to be provided. A similar attitude may explain the connection between folic acid knowledge and pregnancy intention. Women intending or planning to conceive may be more preoccupied with pregnancy-related health issues.

Some limitations of our study should be acknowledged. Despite the generally high participation among ethnic groups, some response bias may still exist to the disadvantage of illiterate and very poorly educated women. If present, however, our prevalence rates of folic acid use and knowledge are an overestimate of the real figures, which in turn emphasises the importance of our findings. We are aware that the advantages attached to measuring language proficiency by self-report are counterbalanced by the possibility of a positive response tendency. Again, this does not alter our conclusions as the observed associations would then be an underestimate of the real associations. Finally, although our study population allowed us to investigate the role of language barriers, we were not able to investigate other cultural factors and barriers on top of language. However, in *post hoc* analyses (results not shown) we observed that folic acid knowledge and use was higher among second generation women (born in the Netherlands), than among first generation women (born elsewhere). These findings imply a minor role of culture: second generation ethnic minority groups, who are educated in the Netherlands, are generally proficient in the Dutch language but only semi-adapted to the Dutch culture, as shifts in cultural orientation and behaviours are gradual and cross generations.[31] The observed reasons for nonuse, as well as the similarity in findings across ethnic groups, additionally indicate that the role of cultural and religious beliefs in this population is not a major one.

In summary, our findings suggest that the primary obstacle for foreign-born (in particular non-Western women) in receiving proper prenatal care – here reflected in the knowledge about and use of folic acid supplements – is the inability to speak and understand the language of the country of residence. Health promotion campaigns addressing these women therefore require linguistically appropriate, easily accessible information. Anticipating this, maternal and child health centres in the Netherlands have introduced ethnic health advisors: specially trained women of foreign origin who educate and inform women from similar ethnic backgrounds in their own language. From a more general perspective, the language education that the Dutch Government has recently made mandatory for all new immigrants is a promising approach, particularly when it integrates information on (prenatal) health and health care.

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Supplementary tables

Table S3.1 Determinants of folic acid knowledge in a multicultural group of pregnant women: forward regression analysis

	Netherlands (n = 5038)	**Surinam (n = 438)**	**Netherlands Antilles (n = 93)**	**Turkey (n = 325)**	**Morocco (n = 558)**	**Ghana (n = 187)**	**Other non-Western country (n = 694)**	**Other Western country (n = 717)**
Determinants	OR[#]	OR	OR	OR	OR	OR	OR	OR
Proficiency in Dutch								
Low	Not offered*	Not offered	Not offered	1	1	1	1	1
Intermediate				2.0 (1.0–4.2)	1.0 (0.6–1.9)	1.5 (0.7–3.5)	1.8 (1.2–2.7)	1.7 (0.9–3.0)
High				16.0 (8.2–31.1)	7.8 (4.8–12.6)	3.7 (1.6–8.4)	7.3 (4.9–11.1)	3.2 (1.9–5.3)
Age (years)								
≤24	0.4 (0.3–0.6)	0.4 (0.2–0.8)		1.0 (0.5–1.9)				0.7 (0.3–1.5)
25–29	1	1		1				1
30–34	3.0 (1.9–4.6)	1.0 (0.5–2.1)		2.6 (1.2–5.7)				2.4 (1.3–4.2)
≥35	2.2 (1.4–3.4)	0.8 (0.4–1.7)		0.6 (0.2–1.7)				2.3 (1.3–4.5)
Education (years)								
0–5	1	1	1		1	1	1	1
6–10	3.0 (2.2–4.0)	4.2 (2.6–7.0)	5.4 (1.6–18.5)		2.0 (1.3–3.0)	2.4 (1.2–5.0)	1.7 (1.1–2.4)	2.2 (1.2–3.8)
≥11	8.7 (5.5–13.8)	9.2 (2.7–31.2)	4.8 (1.4–16.4)		2.6 (1.1–6.2)	11.9 (2.8–51.3)	4.7 (2.8–7.7)	2.4 (1.3–4.5)
Pregnancy experience								
Parity ≥ 1								
Parity = 0								
Pregnancy intention								
No	1	1						
Yes	2.1 (1.4–3.1)	2.0 (1.1–3.7)						

[#] Odds ratio and 95% confidence interval; presented only for determinants that are significantly associated with the outcome variable, adjusted for the other significant determinants.

** Not offered as possible determinant in the regression analysis as the Netherlands, Surinam, and Netherlands Antilles have Dutch as official native language.*

regel 39 regel 38 regel 37 regel 36 regel 35 regel 34 regel 33 regel 32 regel 31 regel 30 regel 29 regel 28 regel 27 regel 26 regel 25 regel 24 regel 23 regel 22 regel 21 regel 20 regel 19 regel 18 regel 17 regel 16 regel 15 regel 14 regel 13 regel 12 regel 11 regel 10 regel 9 regel 8 regel 7 regel 6 regel 5 regel 4 regel 3 regel 2 regel 1

Table S3.2 Determinants of folic acid supplement use in a multicultural group of pregnant women: forward regression analysis

	Netherlands (n = 5038)	**Surinam (n = 438)**	**Netherlands Antilles (n = 93)**	**Turkey (n = 325)**	**Morocco (n = 558)**	**Ghana (n = 187)**	**Other non-Western country (n = 694)**	**Other Western country (n = 717)**
Determinants	OR[#]	OR	OR	OR	OR	OR	OR	OR
Proficiency in Dutch	Not offered*	Not offered	Not offered					
Low								
Intermediate								
High								
Age (years)								
≤24	0.5 (0.4–0.7)						0.6 (0.3–1.2)	0.4 (0.2–0.8)
25–29	1						1	1
30–34	1.9 (1.5–2.5)						2.6 (1.5–4.4)	1.3 (0.8–2.4)
≥35	1.8 (1.4–2.4)						1.6 (0.8–3.0)	1.6 (0.8–2.9)
Education (years)								
0–5	1			1				1
6–10	1.6 (1.2–2.0)			3.2 (1.5–6.7)				2.0 (1.1–3.5)
≥11	2.0 (1.5–2.7)			6.9 (1.7–28.1)				1.8 (1.0–3.4)
Pregnancy experience								
Parity ≥ 1	1	1			1		1	1
Parity = 0	2.5 (2.0–3.0)	1.8 (1.2–2.8)			2.1 (1.3–3.4)		2.5 (1.6–4.0)	2.1 (1.4–3.4)
Pregnancy intention								
No	1	1						
Yes	3.2 (2.3–4.4)	2.1 (1.1–3.9)						
Folic acid knowledge								
No	1	1	1	1	1	1	1	1
Yes	13.1 (9.5–18.2)	13.5 (7.2–25.3)	10.9 (3.8–31.8)	38.1 (17.2–84.5)	18.4 (10.8–31.4)	22.2 (9.0–54.6)	42.0 (25.7–68.6)	17.4 (10.1–29.8)

[#] *Odds ratio and 95% confidence interval; presented only for determinants that are significantly associated with the outcome variable, adjusted for the other significant determinants.*

* *Not offered as possible determinant in the regression analysis as the Netherlands, Surinam, and Netherlands Antilles have Dutch as official native language.*

References

1. Kogan MD, Kotelchuck M, Johnson S. Racial differences in late prenatal care visits. J Perinatol 1993;13:14–21.
2. Rowe RE, Garcia J. Social class, ethnicity and attendance for antenatal care in the United Kingdom: a systematic review. J Public Health Med 2003;25:113–9.
3. Herbst MA, Mercer BM, Beazley D, Meyer N, Carr T. Relationship of prenatal care and perinatal morbidity in low-birth-weight infants. Am J Obstet Gynecol 2003;189:930–3.
4. Ryan GM, Sweeney PJ, Solola AS. Prenatal care and pregnancy outcome. Am J Obstet Gynecol 1980;137:876–81.
5. Vintzileos AM, Ananth CV, Smulian JC, Scorza WE, Knuppel RA. The impact of prenatal care on neonatal deaths in the presence and absence of antenatal high-risk conditions. Am J Obstet Gynecol 2002;186:1011–6.
6. Mayberry RM, Mili F, Ofili E. Racial and ethnic differences in access to medical care. Med Care Res Rev 2000;57(Suppl 1):108–45.
7. Stronks K, Ravelli AC, Reijneveld SA. Immigrants in the Netherlands: equal access for equal needs? J Epidemiol Community Health 2001;55:701–7.
8. Rowe RE, Garcia J, Davidson LL. Social and ethnic inequalities in the offer and uptake of prenatal screening and diagnosis in the UK: a systematic review. Public Health 2004;118:177–89.
9. Gavin NI, Adams EK, Hartmann KE, Benedict MB, Chireau M. Racial and ethnic disparities in the use of pregnancy-related health care among Medicaid pregnant women. Matern Child Health J 2004;8:113–26.
10. Solis JM, Marks G, Garcia M, Shelton D. Acculturation, access to care, and use of preventive services by Hispanics: findings from HHANES 1982-84. Am J Public Health 1990;80(Suppl):11–9.
11. Fiscella K, Franks P, Doescher MP, Saver BG. Disparities in health care by race, ethnicity, and language among the insured: findings from a national sample. Med Care 2002;40:52–9.
12. Derose KP, Baker DW. Limited English proficiency and Latinos' use of physician services. Med Care Res Rev 2000;57:76–91.
13. Bigby J, Perez-Stable E. The challenges of understanding and eliminating racial and ethnic disparities in health. J Gen Intern Med 2004;19:201–3.
14. Woloshin S, Schwartz LM, Katz SJ, et al. Is language a barrier to the use of preventive services? J Gen Intern Med 1997;12:472–7.
15. Free C, White P, Shipman C, Welch HG. Access to and use of out-of-hours services by members of Vietnamese community groups in South London: a focus group study. Fam Pract 1999;16:369–74.
16. Botto LD, Lisi A, Robert-Gnansia E, et al. International retrospective cohort study of neural tube defects in relation to folic acid recommendations: are the recommendations working? BMJ 2005;330:571.
17. Bakker MK, Cornel MC, de Walle HE. Kennis over en gebruik van periconceptioneel foliumzuur onder allochtone en westerse vrouwen, na de publiekscampagne in 1995 (Awareness and periconceptional use of folic acid among non-western and western women in the Netherlands following the 1995 publicity campaign). Ned Tijdschr Geneeskd 2003;147:2426–30 (in Dutch).

18. Howell SR, Barnett AG, Underwood MR. The use of pre-conceptional folic acid as an indicator of uptake of a health message amongst white and Bangladeshi women in Tower Hamlets, east London. Fam Pract 2001;18:300–3.
19. van der Pal-de Bruin KM, de Walle HE, Jeeninga W, et al. The Dutch 'Folic Acid Campaign'--have the goals been achieved? Paediatr Perinat Epidemiol 2000;14:111–7.
20. Keij I. Standaarddefinitie allochtonen (Standard definition ethnic minorities). Index (Central Bureau of Statistics-The Netherlands) 2000;(10):24–25 (in Dutch).
21. Vollset SE, Lande B. Knowledge and attitudes of folate, and use of dietary supplements among women of reproductive age in Norway 1998. Acta Obstet Gynecol Scand 2000;79:513–9.
22. Morin P, De Wals P, St-Cyr-Tribble D, Niyonsenga T, Payette H. Pregnancy planning: a determinant of folic acid supplements use for the primary prevention of neural tube defects. Can J Public Health 2002;93:259–63.
23. Rosenberg KD, Gelow JM, Sandoval AP. Pregnancy intendedness and the use of periconceptional folic acid. Pediatrics 2003;111:1142–5.
24. van der Pal-de Bruin KM, de Walle HE, de Rover CM, et al. Influence of educational level on determinants of folic acid use. Paediatr Perinat Epidemiol 2003;17:256–63.
25. Scott P. Lay beliefs and the management of disease amongst West Indians with diabetes. Health Soc Care Community 1998;6:407–19.
26. Morgan M. The significance of ethnicity for health promotion: patients' use of anti-hypertensive drugs in inner London. Int J Epidemiol 1995;24(suppl 1):S79–84.
27. Lawrence T. A stage-based approach to behaviour change. In: Perkins ER, Simnett I, Wright L, eds. Evidence-based health promotion. Chichester: Wiley, 1999:64–75.
28. Blondel B, Marshall B. Poor antenatal care in 20 French districts: risk factors and pregnancy outcome. J Epidemiol Community Health 1998;52:501–6.
29. Celik Y, Hotchkiss DR. The socio-economic determinants of maternal health care utilization in Turkey. Soc Sci Med 2000;50:1797–806.
30. van Vree F, van der Kemp S, Foets M. Foliumzuurgebruik en gezond gedrag bij zwangerschap: een onderzoek naar determinanten van gedrag bij zwangere vrouwen met een verschillende culturele achtergrond en een verschillende sociaal-economische status (Folic acid use and health behaviour in pregnancy: a study into determinants of behaviour of pregnant women with different cultural backgrounds and socio-economic status). Leiden, The Netherlands: Research voor Beleid, 2003 (in Dutch).
31. Perez W, Padilla AM. Cultural orientation across three generations of Hispanic adolescents. Hisp J Behav Sci 2000;22:390–8.

Chapter 4

ETHNIC DIFFERENCES IN EARLY PREGNANCY MATERNAL N-3 AND N-6 FATTY ACID CONCENTRATIONS NOT EXPLAINED BY FISH CONSUMPTION

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Submitted

Abstract

Ethnicity-related differences in maternal n-3 and n-6 fatty acid status may be relevant to ethnic disparities in birth outcomes observed worldwide. This study explored differences in early pregnancy n-3 and n-6 fatty acid concentrations between Dutch and ethnic minority pregnant women in Amsterdam, the Netherlands, and additionally assessed the role of n-3 fatty acid intake from fish in explaining differences in eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3), dihomo-γ-linolenic acid (DGLA, 20:3n-6), and arachidonic acid (AA, 20:4n-6). Data were derived from the Amsterdam Born Children and their Development cohort (inclusion January 2003 – March 2004). Compared to Dutch women (n = 2459), Surinamese (n = 292), Antillean (n = 64), Turkish (n = 168), and Moroccan (n = 244) women had generally lower concentrations of n-3 fatty acids but higher concentrations of n-6 fatty acids (general linear model, $P < 0.001$). Ghanaian women (n = 57) had higher EPA and DHA concentrations, but generally lower n-6 fatty acid concentrations ($P < 0.001$). Although differences were most pronounced in Turkish and Ghanaian women, who reported the lowest and highest fish consumption respectively, adjustment for fish intake attenuated the differences in EPA, DHA, DGLA, and AA concentrations only modestly. The observed ethnic differences in n-3 and n-6 fatty acid patterns may reflect metabolic rather than intake variation, which warrants further research.

Introduction

Throughout the world, large ethnic disparities in the birth weight distribution can be observed, with the lowest birth weights and highest proportions of intrauterine growth restriction usually being found in minority populations.[1-3] One of the key factors put forward as contributing to these disparities is maternal nutrition.[4]

In the past few decades, the role of the maternal n-3 and n-6 long-chain polyunsaturated fatty acid (LC-PUFA) status in pregnancy has received increasing attention. As structural components of cell membranes and precursors of prostaglandins and other eicosanoids, LC-PUFAs – and particularly eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3), dihomo-γ-linolenic acid (DGLA, 20:3n-6), and arachidonic acid (AA, 20:4n-6) – are considered to be indispensable for adequate fetal growth and development.[5,6] DGLA, AA, and EPA are precursors of the prostaglandins 1, 2, and 3 series respectively, a series of hormone-like substances involved in a range of pregnancy-related processes that include placental blood flow, cervix-ripening, and initiation of parturition.[5,7] DHA, as well as AA, are major components of neural tissue in particular.[8,9]

As the human body cannot synthesize LC-PUFAs *de novo*, these fatty acids need to be derived from the diet, either directly or indirectly. The latter, indirect, pathway involves the endogenous formation of LC-PUFAs from their precursor fatty acids α-linolenic acid (18:3n-3) and linoleic acid (18:2n-6), a complex metabolic pathway in which the n-3 and n-6 fatty acids compete for the same desaturation and elongation enzymes (**Figure 4.1**).[6,9] In the habitual Western diet, the intake of linoleic acid largely exceeds that of α-linoleic acid, and because of this the endogenous production of the n-6 LC-PUFAs is generally favored over that of EPA and DHA. However, if dietary intake of EPA and DHA is high, this effect is to some extent counterbalanced, resulting in reduced concentrations of n-6 LC-PUFAs.[10-13]

While the evidence presented above suggests a role for the n-3 and n-6 fatty acids in the ethnicity-related disparities in perinatal health, few studies have investigated ethnicity-related differences in maternal fatty acid status or how these relate to dietary intake. Therefore, the purpose of the present study was twofold: (i) to explore the within-country ethnic differences in maternal n-3 and n-6 fatty acid status, and (ii) to explore to what extent differences in fish consumption could explain the differences in the major functional LC-PUFAs EPA, DHA, DGLA, and AA. Data were derived from a large, unselected multi-ethnic cohort in Amsterdam, the Netherlands. In this population, large disparities in birth weight have been observed that could not be explained by conventional risk factors.[14]

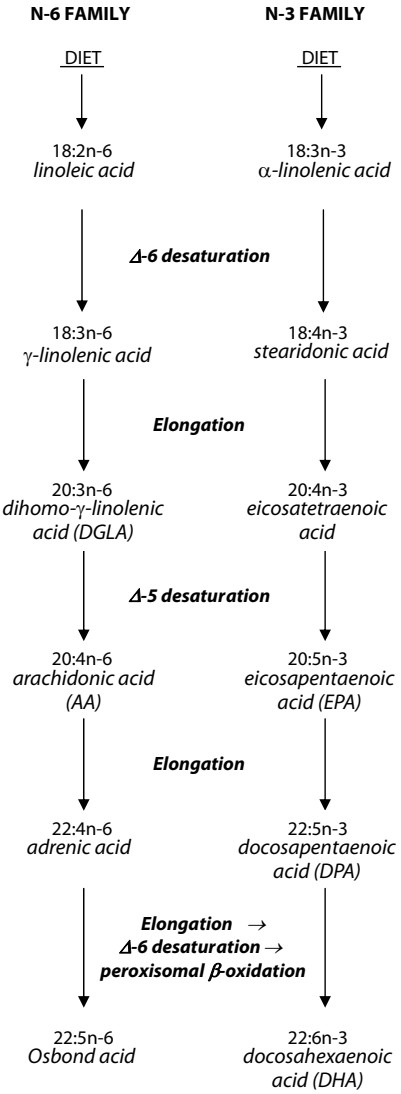

Figure 4.1 Schematic presentation of the preferred metabolic pathway of the n-3 and n-6 fatty acids.

Methods

Study population and design

The Amsterdam Born Children and their Development (ABCD) study is a prospective community-based cohort study that examines the relationship between maternal lifestyle and psychosocial conditions during pregnancy and the child's health at birth as well as in later

life. The essentials of the study design have been described previously.[15] In short, between January 2003 and March 2004, all pregnant women living in Amsterdam were invited to enroll in the ABCD study during their first prenatal visit to their obstetric care provider (around the 12th week of gestation). They were requested to complete a questionnaire, covering sociodemographic data, obstetric history, lifestyle, dietary habits, and psychosocial factors. The questionnaire was available in Dutch as well as in English, Turkish, and Arabic for immigrant women. In addition, women were invited to participate in the ABCD biomarker study. For this study, an additional blood sample was taken during routine blood collection for screening purposes following the first prenatal check-up.

Of the 12 373 pregnant women invited to participate, 8266 returned the pregnancy questionnaire (response rate 67%). Of these respondents, 53% (n = 4389) participated in the biomarker study. Approval of the study was obtained from the Medical Ethical Committees of participating hospitals and the Registration Committee of Amsterdam, and participants gave written informed consent.

Blood collection and analytical methods

For each participant of the biomarker study, a blood sample was taken in a 10 mL EDTA(K2) Vacutainer (Becton and Dickinson BV, Alphen aan de Rijn, the Netherlands) and sent to the Regional Laboratory of Amsterdam for processing. The samples were sent by courier or overnight mail in special envelopes, enabling processing within 28 hours of sampling. A previous study of our group demonstrated that this delay did not compromise the validity of the biomarkers measured.[16] At the laboratory, plasma was prepared by centrifugation (1600 x *g* for 10 min at room temperature) and stored as 1-mL aliquots at −80°C until analysis.

Fatty acid analysis was performed at the Analytical Biochemical Laboratory (ABL, Assen, the Netherlands) using a previously described methodology.[17,18] In short, after the addition of an internal standard (1,2-dinonadecanoyl-*sn*-glycero-3-phosphocholine) and 10-heptadecenoic acid (17:1) to check for carry-over of free fatty acids during the isolation procedure, plasma lipid extracts were prepared by a modified Folch extraction method[19] and phospholipids were isolated by solid-phase extraction on aminopropyl-silica columns (500 mg/3 mL, Varian, Palo Alto, CA, USA).[20] Subsequently, the phospholipids were hydrolyzed and the resulting fatty acids methylated with boron trifluoride-methanol.[21] Finally, the fatty acid methyl esters were quantified by capillary gas chromatography with flame ionization detection (HP5890 series II, Hewlett Packard, Palo Alto, CA, USA), using a polar and a non-polar column (BPx70 and BP1, respectively; SGE Analytical Science Pty. Ltd, Ringwood, Victoria, Australia). The oven temperature was programmed to begin at 160°C for 4 minutes, and then to increase to 200°C by 6.0 °C/min. After 3 minutes, the temperature was further increased to 260°C at a rate of 7 °C/min, and kept constant for 2.34 min. The injector temperature was kept at 250°C and the detector temperature at 300°C.

Absolute amounts of fatty acids (mg/L plasma) were quantified on the basis of recovery from

the internal standard and calculated in relative values (percent of total plasma phospholipid-associated fatty acids). The present study focused only on the n-3 and n-6 fatty acids and included the n-3 fatty acids α-linolenic acid (18:3n-3), eicosatetraenoic acid (20:4n-3), EPA (20:5n-3), docosapentaenoic acid (DPA, 22:5n-3), and DHA (22:6n-3); and the n-6 fatty acids linoleic acid (18:2n-6), DGLA (20:3n-6), AA (20:4n-6), adrenic acid (22:4n-6), and Osbond acid (22:5n-6). Interassay coefficients of variation for these fatty acids varied from ≤22% for 20:4n-3 (the fatty acid with the lowest concentration) to ≤2% for 18:2n-6 (the fatty acid with the highest concentration). Stearidonic acid (18:4n-3) and γ-linolenic acid (18:3n-6) were not included, as their concentrations were <0.1% of total fatty acids.

Questionnaire measurements

Information on ethnic origin, habitual fish and fish oil intake, and potential confounding factors was obtained from the pregnancy questionnaire. We defined ethnic origin by the woman's country of birth or that of her mother, so that second generation women could also be included in the ethnic minority groups.[14] A total of seven ethnic groups were defined: Dutch (reference group), Surinamese, Antillean, Turkish, Moroccan, Ghanaian, and other ethnic origin.

Habitual fish and fish oil consumption was addressed by four frequency questions adapted from the Danish fish frequency questionnaire of Olsen and Secher.[22] Women were asked to indicate how often during their pregnancy they had eaten (a) fish as part of a hot meal; (b) bread or toast with fish, (c) salad (such as a green salad or pasta salad) with fish, and (d) fish oil as a supplement or cooking oil. Predefined response categories were: never, less than once a month, 1–3 times a month, 1–2 times a week, 3–6 times a week, and every day. Assuming these categories to correspond to 0, 0.5, 2, 4, 20, and 28 servings per 28 days respectively,[22] we combined the responses on the four questions into one summarized measure, representing a total number of fish and fish oil servings per week. Subsequently, three frequency groups were defined in such way that each group was of a reasonable size and that intake frequency increased progressively: (1) <1 serving per week, (2) 1–1.9 servings per week, and (3) ≥2 servings per week.

Five physiologic and lifestyle-related variables previously reported to influence fatty acid dynamics were considered potential confounders. These included maternal age (years),[23] parity (0, 1, ≥2),[24,25] pregravid body mass index (BMI, kg/m^2) as based on self-reported height and weight,[26] and smoking and alcohol consumption during pregnancy (self-reported previous week's behavior, recoded into yes, no).[27,28] For height and weight, missing values (3.3% and 9.8% respectively) were imputed by means of a random imputation procedure using linear regression,[29] which accounted for the differences among the ethnic groups.

Statistical analysis

Fatty acid results were available for 4336 of the 4389 participants. We excluded all respondents

with known diabetes (n = 26) or hypertension at the time of blood sampling (n = 152), as well as all respondents with missing information on fish or fish oil consumption (n = 25). Restriction to the six main ethnic groups (Dutch, Surinamese, Antillean, Turkish, Moroccan, and Ghanaian) provided the final sample for analysis of n = 3284.

First, differences in the distribution of relevant maternal characteristics and the habitual fish and fish oil intake between the Dutch and ethnic minority groups were described and tested with the Chi-square test for categorical variables and ANOVA for continuous variables. Second, ethnic differences in fatty acid concentrations were explored using the general linear model function in SPSS, which tests both the overall association between ethnic origin and fatty acid concentrations as well as the fatty acid specific ethnic differences.[30] Finally, the contribution of maternal fish consumption to the differences in maternal concentrations of EPA, DHA, DGLA, and AA was explored by extending the basic general linear model (model 1, including only ethnicity) first with the potential confounders (model 2) and then with the fish frequency measure (model 3). We used the model estimated means to calculate the relative (i.e., percentage) differences between the ethnic minority groups and the Dutch reference group and assumed the changes in relative differences across model 2 and 3 to indicate the contribution of fish consumption to these differences. When necessary, transformations were applied to obtain more symmetrical distributions and improve the normality of the residuals in the various models (18:3n-3, 20:4n-3, 22:5n-6: square root transformation; EPA (20:5n-3): log transformation). For 22:4n-6, 22:5n-6, 18:3n-3, 20:4n-3 and EPA, the measurements included zero values (<0.5% of cases), which were replaced by half of the value of the lowest measured value.

To assess the influence of changes in the fatty acid concentrations that normally occur during pregnancy,[17] all models were repeated with gestational age at blood sampling (based on ultrasound, or if unavailable, on the time of the last menstrual period) as covariate. However, as the order of magnitude of the differences were similar, we preferred to present the most parsimonious models (results for the standardized models available from the authors upon request). Because of the multiple comparisons made, associations were considered significant at $P < 0.01$. All analyses were conducted in SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

In the present analysis, 25% of the study population was of non-Dutch descent. The baseline characteristics of the ethnic groups are presented in **Table 4.1**. Overall, non-Dutch women had their prenatal check-up including the blood sampling at a later gestational age than the Dutch women (≥14.6 vs. 12.9 weeks). Between the ethnic groups, significant differences existed in the distribution of maternal age, parity, BMI, smoking habits, and alcohol consumption ($P <$ 0.001). Dutch women were generally older than women of non-Dutch descent; on average,

the youngest mothers were of Turkish descent. Antillean and Dutch women were more often nulliparous and had a lower BMI on average. Women from Ghana were more often multiparous (i.e., parity ≥ 2) and had the highest BMI. Turkish women reported smoking during pregnancy more often than Dutch women, while Moroccan, Antillean, and Ghanaian women rarely smoked. Alcohol consumption was most common among Dutch women.

Table 4.1 Characteristics of the study population according to ethnic origin[a]

Characteristic	Ethnic group						*P*-value[b]
	Dutch (n = 2459)	Surinamese (n = 292)	Antillean (n = 64)	Turkish (n = 168)	Moroccan (n = 244)	Ghanaian (n = 57)	
Gestational age at blood sampling (wk)	12.9 ± 2.7	14.8 ± 4.4*	14.7 ± 4.9*	14.6 ± 3.5*	15.4 ± 4.4*	14.8 ± 4.9*	<0.001
unknown (%)	0.0	1.6	0.6	0.0	0.0	0.5	
Maternal age (y)	31.9 ± 4.0	28.8 ± 6.3*	28.4 ± 6.4*	25.7 ± 5.0*	27.3 ± 5.2*	31.5 ± 5.7	<0.001
Parity (%)							
0	60.9	49.0*	75.0	46.4*	48.0*	28.1*	<0.001
1	32.1	28.4	18.8	33.9	25.8	28.1	
≥2	7.0	22.6	6.3	19.6	26.2	43.9	
Pregravid BMI (kg/m^2)	22.4 ± 3.2	23.6 ± 4.3*	23.1 ± 4.5	23.6 ± 4.0*	24.7 ± 4.4*	26.3 ± 4.7*	<0.001
Smoking (%)	9.8	15.8[§]	3.1	23.8*	2.5*	1.8	<0.001
Alcohol consumption (%)	31.1	11.3*	17.2	0.6*	0.4*	10.5*	<0.001

[a] *Values are means ± SD or %.*

[b] *Test for differences between groups; χ^2 statistic for categorical variables, ANOVA for continuous variables.*

** Significantly different distribution from Dutch ethnic group, P < 0.001.*

[§] *Significantly different distribution from Dutch ethnic group, P < 0.01.*

Details of maternal fish consumption are presented in **Table 4.2**. Overall, nearly 20% of the women reported consuming a serving of fish or fish oil at least twice a week, while just over 45% reported consuming fish or fish oil less than once a week. While fish or fish oil consumption was similar among Dutch, Surinamese, Antillean, and Moroccan women, lower consumption was found among Turkish women and higher consumption among Ghanaian women (respectively 13% and 56% reported consuming a serving of fish or fish oil at least twice a week).

Table 4.3 shows the maternal fatty acid concentrations in plasma phospholipids for each ethnic group separately; for those fatty acids with a skewed distribution, the median and interquartile range is given in addition to the mean and standard deviation. For both n-3 and n-6 fatty acids, considerable ethnicity-related differences were observed (Pillai's trace criterion for multiple outcomes $P < 0.001$).

Table 4.2 Fish and fish oil consumption according to ethnic origin[a]

Fish and fish oil consumption	Ethnic group						*P*-value[b]
	Dutch (n = 2459)	Surinamese (n = 292)	Antillean (n = 64)	Turkish (n = 168)	Moroccan (n = 244)	Ghanaian (n = 57)	
Hot meal with fish (%)							
Never	9.4	7.5	14.1	28.6*	5.7	1.8*	<0.001
<1 per month	19.9	26.4	25.0	32.7	21.7	8.8	
1–3 times per month	42.3	41.8	37.5	25.0	37.3	21.1	
≥1 per week	28.3	24.3	23.4	13.7	35.2	68.4	
Salad with fish (%)							
Never	44.2	60.3*	54.7	64.9*	37.7*	35.1*	<0.001
<1 per month	31.9	20.2	20.3	18.5	23.4	12.3	
1–3 times per month	19.4	12.0	17.2	8.9	20.9	21.1	
≥1 per week	4.5	7.5	7.8	7.7	18.0	31.6	
Fish sandwich (%)							
Never	28.1	27.4	34.4	60.7*	21.7*	50.9*	<0.001
<1 per month	28.8	28.8	21.9	17.9	20.1	14.0	
1–3 times per month	31.2	28.4	29.7	12.5	29.1	15.8	
≥1 per week	11.9	15.4	14.1	8.9	29.1	19.3	
Fish oil (%)							
Never	89.6	89.4	89.1	93.5	78.7*	52.6*	<0.001
<1 per month	6.5	7.2	7.8	4.2	8.6	7.0	
1–3 times per month	1.8	2.1	1.6	0.6	5.7	14.0	
≥1 per week	2.0	1.4	1.6	1.8	7.0	26.3	
Overall frequency of fish and fish oil consumption (%)							
<1 serving per week	45.5	48.6	57.8	69.0*	33.6*	17.5*	<0.001
1–1.9 servings per week	36.7	32.5	25.0	17.9	31.1	26.3	
≥2 servings per week	17.7	18.8	17.2	13.1	35.2	56.1	

[a] *Values are percentages.*

[b] *χ^2 statistic for differences between groups.*

** Significantly different distribution from Dutch ethnic group, P < 0.001.*

Table 4.3 Maternal n-3 and n-6 fatty acid concentrations in plasma phospholipids according to ethnic origin[a]

Fatty acid concentration (% of total fatty acids)	**Ethnic group**						***P*-value[b]**
	Dutch (n = 2459)	Surinamese (n = 292)	Antillean (n = 64)	Turkish (n = 168)	Moroccan (n = 244)	Ghanaian (n = 57)	
n-3 fatty acids							
18:3n-3	0.19 ± 0.07 *0.18 (0.14–0.23)*	0.15 ± 0.07* *0.14 (0.10–0.19)*	0.17 ± 0.06 *0.17 (0.12–0.20)*	0.13 ± 0.06* *0.12 (0.09–0.17)*	0.14 ± 0.07* *0.14 (0.10–0.18)*	0.15 ± 0.06* *0.14 (0.11–0.19)*	<0.001
20:4n-3	0.15 ± 0.06 *0.14 (0.11–0.18)*	0.09 ± 0.05* *0.08 (0.06–0.12)*	0.12 ± 0.05* *0.11 (0.07–0.16)*	0.09 ± 0.05* *0.08 (0.05–0.11)*	0.09 ± 0.05* *0.08 (0.06–0.10)*	0.11 ± 0.07* *0.09 (0.07–0.13)*	<0.001
20:5n-3 (EPA)	0.68 ± 0.39 *0.58 (0.44–0.77)*	0.45 ± 0.38* *0.36 (0.26–0.52)*	0.52 ± 0.47* *0.42 (0.31–0.59)*	0.33 ± 0.28* *0.26 (0.18–0.37)*	0.42 ± 0.27* *0.34 (0.27–0.46)*	1.38 ± 0.91* *1.02 (0.72–1.88)*	<0.001
22:5n-3 (DPA)	0.77 ± 0.17	0.63 ± 0.16*	0.70 ± 0.18*	0.56 ± 0.16*	0.57 ± 0.15*	0.89 ± 0.25*	<0.001
22:6n-3 (DHA)	4.81 ± 1.05	4.77 ± 0.92	4.80 ± 1.00	3.80 ± 0.95*	4.69 ± 0.93	6.76 ± 1.13*	<0.001
n-6 fatty acids							
18:2n-6	18.78 ± 2.40	19.25 ± 2.44§	19.13 ± 2.10	20.62 ± 2.70*	19.99 ± 2.45*	17.78 ± 2.54§	<0.001
20:3n-6 (DGLA)	3.50 ± 0.72	3.12 ± 0.69*	3.23 ± 0.61§	3.63 ± 0.79	3.29 ± 0.68*	2.51 ± 0.60*	<0.001
20:4n-6 (AA)	9.07 ± 1.48	10.33 ± 1.76*	9.72 ± 1.63§	9.70 ± 1.70*	9.80 ± 1.84*	9.96 ± 1.54*	<0.001
22:4n-6	0.37 ± 0.10	0.45 ± 0.12*	0.42 ± 0.11*	0.45 ± 0.15*	0.40 ± 0.10*	0.30 ± 0.10*	<0.001
22:5n-6	0.36 ± 0.14 *0.33 (0.26–0.43)*	0.44 ± 0.17* *0.42 (0.31–0.53)*	0.41 ± 0.16§ *0.37 (0.29–0.50)*	0.54 ± 0.21* *0.51 (0.39–0.64)*	0.38 ± 0.15§ *0.36 (0.28–0.46)*	0.25 ± 0.12* *0.22 (0.17–0.28)*	<0.001

EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid.

[a] Values are means ± SD; for skewed distributions, the median (interquartile range) is also presented in italics .

[b] General linear model; ANOVA statistics for fatty acid-specific difference between groups following the significant overall association between ethnicity and fatty acid concentrations (Pillai's trace criterion; P < 0.001).

** Significantly different distribution from Dutch ethnic group, P < 0.001.*

§ Significantly different distribution from Dutch ethnic group, P < 0.01.

Compared to the Dutch women, all ethnic minority groups had lower concentrations of α-linolenic acid (18:3n-3) and its derivative eicosatetraenoic acid (20:4n-3). For Surinamese, Antillean, and Moroccan women concentrations of EPA (20:5n-3) and DPA (22:5n-3) were also (significantly) lower. The lowest concentrations of EPA and DPA, as well as DHA (22:6n-3) (the three fatty acids found primarily in fish and fish oil) were observed for the Turkish women; on contrast, Ghanaian women were found to have the highest concentrations.

Ethnic patterns were more complex for the n-6 fatty acids. Surinamese, Antillean, Turkish, and Moroccan women all showed higher concentrations of linoleic acid (18:2n-6) and its longer-chain derivatives AA (20:4n-6), adrenic acid (22:4n-6), and Osbond acid (22:5n-6), but lower or comparable concentrations of DGLA (20:3n-6). Interestingly, the Turkish women showed the largest deviation from the Dutch group for linoleic acid, Osbond acid, and adrenic acid, but the smallest deviation for AA and DGLA. The pattern for Ghanaian women was again different: they showed lower concentrations of DGLA as well as linoleic acid, adrenic acid, and Osbond acid, but higher concentrations of AA.

Adjustment for gestational age at blood sampling did not alter the size of the model coefficients (results not shown), but did affect the significance levels; for 5 of the 44 associations, this resulted in *P*-values > 0.01.

Figure 4.2 compares the observed differences in EPA, DHA, DGLA, and AA concentrations across the ethnic minority groups. Results are presented as relative differences (i.e., expressed in percentage difference) from the Dutch concentrations, with models 1 to 3 representing the increasing levels of adjustment. For the fish fatty acids EPA and DHA the figure reveals the extreme position of Ghanaian and Turkish women vs. Dutch women, showing differences of +88% and −55% for EPA, and differences of +41% and −21% for DHA respectively. For the n-6 fatty acids, differences across the ethnic minority groups were less pronounced. The changes across the models showed a modest attenuation of the Dutch – Ghanaian differences in EPA and DHA concentrations by fish and fish oil consumption, but a minor influence of this measure in other group comparisons.

Discussion

In this observational study, we found distinct patterns of maternal fatty acid concentrations in plasma phospholipids across ethnic groups, with the Ghanaian and Turkish ethnic groups deviating most from the Dutch reference group. While we assumed the consumption of fish and fish oil to be relevant to the concentrations of the n-3 long-chain derivatives EPA and DHA, as well as to levels of the n-6 LC-PUFAs DGLA and AA,[13] adjustment for fish intake apparently did not affect the differences in these LC-PUFAs to a relevant degree. This was perhaps to be expected for the ethnic groups that reported intake levels comparable to the Dutch, but not for those who deviated.

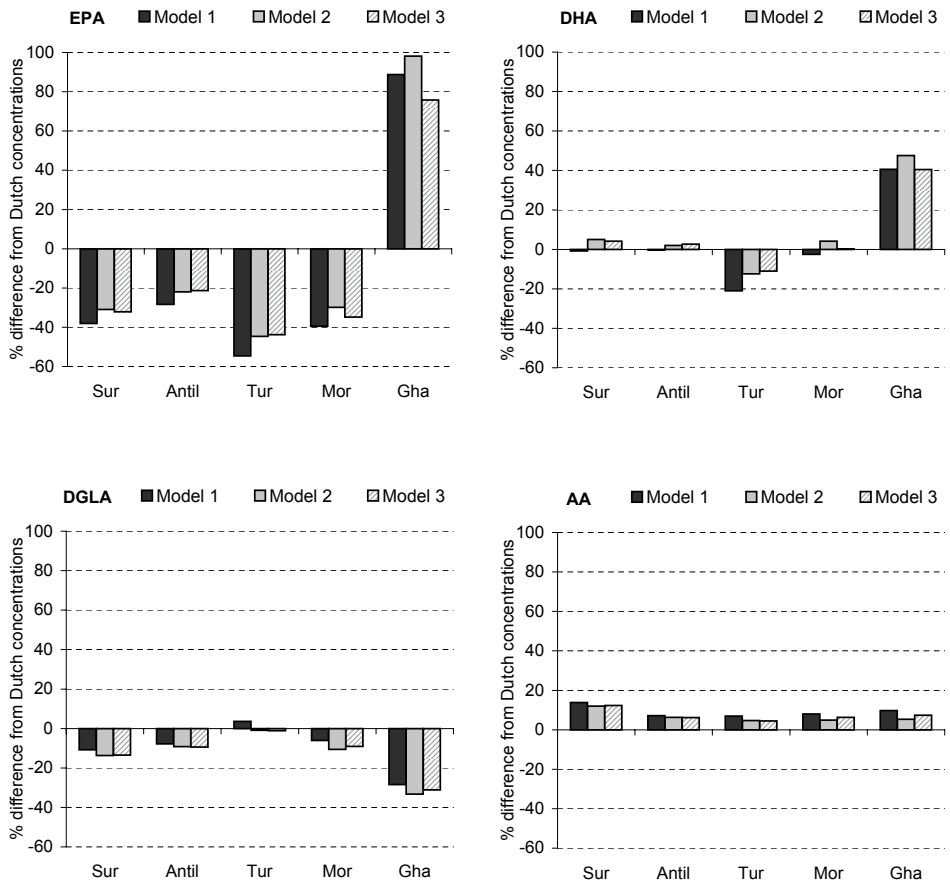

Figure 4.2 EPA, DHA, DGLA, and AA concentrations in the five main ethnic minority groups compared to the Dutch reference group. Concentrations are expressed as percentage difference from Dutch concentrations. *EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid. Sur, Surinamese; Antil, Antillean; Tur, Turkish; Mor, Morrocan; Gha, Ghanaian. Model 1: crude (not adjusted); model 2: as model 1 + adjustment for maternal age, parity, pregravid BMI, smoking, and alcohol consumption; model 3: as model 2 + adjustment for fish and fish oil consumption.*

To our knowledge, only one previous study has examined ethnicity-related differences in maternal fatty acid concentrations in early pregnancy, applying a cross-country rather than a within-country comparison.[31] In that study, large differences in n-3 and n-6 fatty acid concentrations were observed particularly between East and West European countries. However, the study did not adjust for differences in the dietary habits assumed to reflect these disparities, such as the higher fish consumption in West European coastal countries compared to countries in Eastern Europe.[32] When we accounted for fish consumption intake in our

study, the explanatory strength of this variable was only minor, suggesting that alternative explanations should be considered.

A first alternative explanation for the observed ethnic differences in LC-PUFA concentrations relates to interethnic differences in intake and subsequent metabolic conversion of the parent essential fatty acids, in particular linoleic acid. A high intake of linoleic acid (which is generally the case in contemporary diets) not only inhibits the conversion of α-linolenic acid to its LC-PUFAs[33] and their incorporation in plasma phospholipids,[34] but also limits its own conversion, a phenomenon known as substrate inhibition.[35] However, to what extent these inhibitions occur may also differ between ethnic groups, depending on the metabolic activities of the enzymes involved (the δ-6 and δ-5 desaturases in particular).[36] In our study, we were unable to measure linoleic acid intake, but we did observe (as a proxy of intake) relatively high concentrations of linoleic acid among Turkish women, and low concentrations among Ghanaian women, which inversely corresponded with their concentrations of EPA, DHA, and AA. *Post hoc* analyses to further explore the role of metabolic differences revealed a significant contrast between Turkish and Ghanaian women in activity of the δ-5 desaturase, as indicated by the ratio between AA and its precursor DGLA[36] (4.19, SD 1.21 for Ghanaian women; 2.82, SD 0.82 for Turkish women). Inclusion of the linoleic acid concentration and its interaction with ethnicity as explanatory variables in the general linear models additionally indicated the presence of ethnic-specific metabolic patterns, in particular for EPA ($P < 0.001$) and AA ($P = 0.047$). However, further research is required to establish these differences and to examine whether they are genetic or rather epigenetic, as would be assumed from the discordance between genetically related groups (i.e., an African ancestry for Ghanaian, Antillean, and Surinamese women; and a Caucasian ancestry for Turkish, Moroccan, and Dutch women).[37]

A potential second explanation of the ethnic disparities in LC-PUFA status involves the pregnancy-specific fatty acid dynamics. Longitudinal studies have reported considerable increases in LC-PUFA concentrations during the course of pregnancy, presumably as the result of a fatty acid mobilization from maternal body stores, such as adipose tissue.[17,38] This process starts shortly after conception, and although we lack information on potential differences in lipolytic activity between these groups as well as on differences in the amount and LC-PUFA content of their adipose tissue, it could explain our results, at least in part.

A final explanation of our observations, and in particular of the poor explanatory strength of fish consumption, relates to the fish frequency questionnaire itself. Our measurement of fish and fish oil consumption was derived from a Danish fish frequency questionnaire,[22] which may have had lower validity in this Dutch multi-ethnic context. However, when we examined the Spearman rank correlation between EPA concentrations and the number of servings per week, the overall correlation (0.334) was well in the range of previous questionnaire-concentration correlations (0.19–0.50),[13] with a nonsignificant correlation only for Ghanaians (0.136). Rather than a validity problem, this heterogeneity of correlations may reflect the metabolic differences indicated earlier.

In conclusion, our results suggest the presence of considerable ethnic disparities in n-3 and n-6 fatty acid concentrations during pregnancy, which, in view of the existing and newly emerging evidence of the role of these nutrients in human growth and development, may be relevant to ethnic disparities in perinatal health. Given the limitations of this observational study, further research into these distinct ethnicity-related fatty acid patterns is warranted, particularly to elucidate the role of intake vs. metabolic differences.

Acknowledgements

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References

1. Troe EJ, Raat H, Jaddoe VW, et al. Explaining differences in birthweight between ethnic populations. The Generation R study. BJOG 2007;114:1557–65.
2. Harding S, Rosato MG, Cruickshank JK. Lack of change in birthweights of infants by generational status among Indian, Pakistani, Bangladeshi, Black Carribean, and Black African mothers in a British cohort study. Int J Epidemiol 2004;33:1279–85.
3. Shiono PH, Rauh VA, Park M, Lederman SA, Zuskar D. Ethnic differences in birthweight: the role of lifestyle and other factors. Am J Public Health 1997;87:787–93.
4. Cohen GR, Curet LB, Levine RJ, et al. Ethnicity, nutrition, and birth outcomes in nulliparous women. Am J Obstet Gynecol 2001;185:660–7.
5. Uauy R, Treen M, Hoffman DR. Essential fatty acid metabolism and requirements during development. Semin Perinatol 1989;13:118–30.
6. Hornstra G. Importance of polyunsaturated fatty acids of the n-6 and n-3 families for early human development. European Journal of Lipid Science and Technology 2001;103:379–89.
7. Allen KG, Harris MA. The role of n-3 fatty acids in gestation and parturition. Exp Biol Med 2001;226:498–506.
8. Uauy R, Calderon F, Mena P. Essential fatty acids in somatic growth and brain development. World Rev Nutr Diet 2001;89:134–60.
9. Innis SM. Perinatal biochemistry and physiology of long-chain polyunsaturated fatty acids. J Pediatr 2003;143(4 suppl):S1–8.
10. van Houwelingen AC, Sørensen JD, Hornstra G, et al. Essential fatty acid status in neonates after fish-oil supplementation during late pregnancy. Br J Nutr 1995;74:723–31.
11. Hjartåker A, Lund E, Bjerve KS. Serum phospholipid fatty acid composition and habitual intake of marine foods registered by a semi-quantitative food frequency questionnaire. Eur J Clin Nutr 1997;51:736–42.
12. Dunstan JA, Mori TA, Barden A, et al. Effects of n-3 polyunsaturated fatty acid supplementation in pregnancy on maternal and fetal erythrocyte fatty acid composition. Eur J Clin Nutr 2004;58:429–37.
13. Williams MA, Frederick IO, Qiu C, et al. Maternal erythrocyte omega-3 and omega-6 fatty acids, and plasma lipid concentrations, are associated with habitual dietary fish consumption in early pregnancy. Clin Biochem 2006;39:1063–70.
14. Goedhart G, van Eijsden M, van der Wal MF, et al. Ethnic differences in term birthweight; the role of constitutional and environmental factors. Paediatr Perinat Epidemiol (in press).
15. van Eijsden M, van der Wal MF, Bonsel GJ. Folic acid knowledge and use in a multi-ethnic pregnancy cohort: the role of language proficiency. BJOG 2006;113:1446–51.
16. van Eijsden M, van der Wal MF, Hornstra G, Bonsel GJ. Can whole-blood samples be stored over 24 hours without compromising stability of C-reactive protein, retinol, ferritin, folic acid and fatty acids in epidemiologic research? Clin Chem 2005;51:230–2.
17. Al MD, van Houwelingen AC, Kester AD, Hasaart TH, de Jong AE, Hornstra G. Maternal essential fatty acid patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status. Br J Nutr 1995;74:55–68.

18. Otto SJ, van Houwelingen AC, Hornstra G. The effect of different supplements containing docosahexaenoic acid on plasma and erythrocyte fatty acids of healthy non-pregnant women. Nutr Res 2000;20:917–27.
19. Hoving EB, Jansen G, Volmer M, van Doormaal JJ, Muskiet FA. Profiling of plasma cholesterol ester and triglyceride fatty acids as their methyl esters by capillary gas chromatography, preceded by a rapid aminopropyl-silica column chromatographic separation of lipid classes. J Chromatogr 1988;434:395–409.
20. Kaluzny MA, Duncan LA, Merritt MV, Epps DE. Rapid separation of lipid classes in high yield and purity bonded phase columns. J Lipid Res 1985;26:135–40.
21. Morrison WR, Smith LM. Preparation of fatty acid methylesters and dimethylacetals from lipids with boron fluoride-methanol. J Lipid Res 1964;5:600–8.
22. Olsen SF, Secher NJ. Low consumption of seafood in early pregnancy as a risk factor for preterm delivery: prospective cohort study. BMJ 2002;324:447–50.
23. Maniongui C, Blond JP, Ulmann L, Durand G, Poisson JP, Bezard J. Age-related changes in Δ6 and Δ5 desaturase activities in rat liver microsomes. Lipids 1993;28:291–7.
24. Al MD, van Houwelingen AC, Hornstra G. Relation between birth order and the maternal and neonatal docosahexaenoic acid status. Eur J Clin Nutr 1997;51:548–53.
25. Levant B, Ozias MK, Carlson SE. Diet (n-3) polyunsaturated fatty acid content and parity affect liver and erythrocyte phospholipid fatty acid composition in female rats. J Nutr 2007;137:2425–30.
26. Warensjö E, Örhvall M, Vessby B. Fatty acid composition and estimated desaturase activities are associated with obesity and lifestyle variables in men and women. Nutr Metab Cardiovasc Dis 2006;16:128–36.
27. Simon JA, Fong J, Bernert JT, Browner WS. Relation of smoking and alcohol consumption to serum fatty acids. Am J Epidemiol 1996;144:325–34.
28. Pawlosky R, Hibbeln J, Wegher B, Sebring N, Salem N. The effects of cigarette smoking on the metabolism of essential fatty acids. Lipids 1999;34(suppl):S287.
29. Allison PD. Missing data. Thousand Oaks, CA: Sage Publications, 2001.
30. Tabachnik B, Fidell L. Using multivariate statistics. 5th ed. Boston, MA: Pearson/Allyn & Bacon, 2007.
31. Otto SJ, van Houwelingen AC, Antal M, et al. Maternal and neonatal essential fatty acid status in phospholipids: an international comparative study. Eur J Clin Nutr 1997;51:232–42.
32. Minda H, Larque E, Koletzko B, Decsi T. Systematic review of fatty acid composition of plasma phospholipids of venous cord blood in full-term infants. Eur J Nutr 2002;41:125–31.
33. Emken EA, Adlof RO, Gulley RM. Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic acid and linolenic acids in young adult males. Biochim Biophys Acta 1994;1213:277–88.
34. Grønn M, Gørbitz C, Christensen E, et al. Dietary n-6 fatty acids inhibit the incorporation of dietary n-3 fatty acids in thrombocyte and serum phospholipids in humans: a controlled dietetic study. Scand J Clin Lab Invest 1991;51:255–63.
35. Cunnane SC. The conditional nature of the dietary need for polyunsaturates: a proposal to reclassify 'essential fatty acids' as 'conditionally-indispensable' or 'conditionally-dispensable' fatty acids. Br J Nutr 2000;84:803–12.
36. Hornstra G, Al MD, Gerrard, JM, Simonis MM. Essential fatty acid status of neonates born to Inuit mothers: comparison with Caucasian neonates and effect of diet. Prostaglandins Leukot Essent Fatty Acids 1992;45:125–30.

37. Risch N, Burchard E, Ziv E, Tang H. Categorization of humans in biomedical research: genes, race and disease. Genome Biol. 2002;3(7):comment2007.

38. Otto SJ, van Houwelingen AC, Badart-Smook A, Hornstra G. Changes in the maternal essential fatty acid profile during early pregnancy and the relation of the profile to diet. Am J Clin Nutr 2001;73:302–7.

Chapter 5

ETHNIC DIFFERENCES IN TERM BIRTH WEIGHT; THE ROLE OF CONSTITUTIONAL AND ENVIRONMENTAL FACTORS

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Abstract

It is not clear to what extent ethnic differences in the term birth weight distribution are constitutional or pathological. This study explored term birth weight heterogeneity between ethnic groups and the explanatory role of constitutional and environmental factors. As part of a prospective cohort study, the Amsterdam Born Children and their Development study, 8266 pregnant women filled out a questionnaire during early pregnancy. Ethnic groups were categorized as: native Dutch group; first and second generation Surinamese, Antillean, Turkish, Moroccan, Ghanaian, and other non-Dutch groups. Only singleton live births with 37.0 or more weeks of gestation and with complete data were included for analysis (n = 7118). We performed linear regression analyses to estimate the association between ethnicity and for gestational age standardized birth weight at term, adjusted for constitutional (fetal sex, parity, maternal age, height) and environmental (education, cohabitation status, maternal BMI, smoking, alcohol consumption, depression, work stress) determinants respectively. Mean birth weight ranged from 3223 g (second generation Surinamese newborns) to 3548 g (Dutch newborns). Adjustment for constitutional factors substantially reduced the ethnic differences in birth weight, while adjustment for environmental factors provided little additional explanation. Surinamese (first generation: regression coefficient *(B)* = −98.3 g, $P < 0.001$; second generation: $B = -159.3$ g, $P < 0.001$), first generation Antillean ($B = -102.0$ g, $P = 0.037$), and Ghanaian ($B = -120.7$ g, $P = 0.001$) newborns remained significantly smaller than Dutch newborns after adjustment for all determinants. Term birth weight differences between Dutch newborns and Turkish, Moroccan, and other non-Dutch newborns were largely explained by constitutional rather than environmental determinants, limiting the need for prevention. Surinamese, Antillean, and Ghanaian (mainly black) newborns remained unexplainably smaller after adjustment, leaving room for both a constitutional or pathological underlying mechanism.

Introduction

For decades, researchers worldwide have investigated the incidence of low birth weight (LBW; <2500 g) and its association with adverse health outcomes.[1] Currently, LBW is considered a poor indicator for health outcomes on the population level.[2] This is because newborns defined as LBW are a mixture of preterm born, growth restricted, and constitutionally small babies; all three groups show quite different infant health outcomes. Wilcox[1] proposed to separate preterm from term births (≥37.0 weeks gestation) when studying health outcomes on the population level. The proportion of preterm births should be used as an indicator of a population's infant mortality risk and the birth weight distribution of the remaining term births as a valid measure of fetal growth. Useful as this distinction may be, term birth weight heterogeneity between groups can still be the result of either pathological growth restriction or a "natural" limited growth potential by constitutional profile.

Worldwide, large ethnic differences in the term birth weight distribution are observed.[1,3] Previous studies explained some differences by adjusting for major determinants, such as smoking.[4] However, from a preventive point of view, it is important to explore to what extent ethnic differences in the birth weight distribution can be explained by two main determinant groups: (a) constitutional determinants (e.g., maternal height), which are difficult to modify determinants that physiologically influence fetal growth in a natural way, or (b) environmental determinants (e.g., smoking), which are more or less modifiable determinants that impair fetal growth.[5] A predominant role for constitutional factors would add to a natural explanation of birth weight heterogeneity, with no need for preventive actions, whereas predominance of environmental factors would suggest acquired pathology with, theoretically, a higher probability of short- and long-term adverse health outcomes.

In the present study we explored, in a large prospective multi-ethnic cohort of pregnant women, (i) term birth weight heterogeneity among ethnic groups, and (ii) the roles of constitutional and environmental factors, using a hierarchical model. Taking advantage of the presence of sufficiently sized groups of first and second generation ethnic minority groups we additionally explored intergenerational differences in the birth weight distribution at term.

Methods

The present study is part of the Amsterdam Born Children and their Development (ABCD) study. The ABCD study is a prospective, unselected, and community-based cohort study, which examines the relationship between maternal lifestyle and psychosocial conditions during pregnancy and the child's health at birth and in later life. The primary focus is on the explanation of the existing ethnic disparities in maternal and child health. Approval of the study was obtained from the Central Committee on Research involving Human Subjects in

the Netherlands, the Medical Ethical Committees of participating hospitals, and from the Registration Committee of the Municipality of Amsterdam.

Study population

Between January 2003 and March 2004, all pregnant women living in Amsterdam were invited to enroll in the ABCD study at their first antenatal visit (around the 12th week of gestation) to participating obstetric care providers (general practitioners, midwives, and hospital gynecologists; overall participation rate 96%). Two weeks after their first antenatal visit a pregnancy questionnaire was mailed to all approached women (n = 12 373), to be returned by prepaid mail. The questionnaire was in Dutch, and accompanied by an English, Turkish, or Arabic copy depending on the woman's country of birth. Reminders were sent two weeks after the initial mailing to improve response. The questionnaire was filled out by 8266 women (response rate 67%). For this study only singleton live births with a pregnancy duration of 37.0 or more weeks were included (n = 7318). We did not exclude the pregnancies with fetal malformations in absence of a specific relation between birth weight in fetal malformations and ethnicity. Overall, 7118 women had complete data on all study variables. Mean birth weight of the excluded term births with incomplete data was not significantly different from the mean birth weight of included cases.

Outcome variable

The primary outcome variable was birth weight (in grams) of neonates born at term (gestational duration ≥ 37.0 weeks). Information on the pregnancy outcomes (birth weight, gestational age, and fetal sex) were obtained from the Youth Health Care registration at the Municipal Health Service in Amsterdam. Duration of pregnancy (in weeks and days) was based on ultrasound or, when unavailable (<10%), on the first day of the last menstrual period (calculation by the obstetric care provider).

Determinants

Ethnicity was based on the country of birth of the pregnant woman and her mother. Country of birth included the following categories, based on the Amsterdam main ethnic populations: The Netherlands, Surinam, The Antilles/Aruba, Turkey, Morocco, Ghana, and other non-Dutch countries. Ethnic minority groups were distinguished into first (born outside the Netherlands) and second (born in the Netherlands, but with a mother born in another country) generation women. Because only two women were classified as second generation Ghanaian, these women were merged with the first generation Ghanaians. The native Dutch group (pregnant woman and her mother born in the Netherlands) was used as the reference group.

Determinants of fetal growth, known to be associated with both birth weight and ethnicity, were collected through the pregnancy questionnaire, except for fetal sex and gestational age

(GA) (see above). Birth weight was standardized for GA (linear and quadratic term) in all logistic regression analyses. We distinguished two groups of determinants: constitutional and environmental determinants. Constitutional determinants were: fetal sex, parity (0 vs. ≥1), maternal age (≤24, 25–34, ≥35 year), and maternal height (cm). Environmental determinants were: educational attainment (years of education after primary school: ≤5, 6–10, ≥11 year), cohabitation status (living together with partner vs. not living together/single), maternal prepregnancy BMI (underweight: BMI < 18 kg/m^2, normal weight: 18–24.9, overweight: 25–29.9, obesity: ≥ 30), smoking (average number of cigarettes per day in early pregnancy: none, <1, 1–5, >5 cigarettes), alcohol consumption during early pregnancy (no vs. yes), depression (a score on the CES-D scale[6] of lower vs. higher than the 90th percentile), and work stress (no paid job, paid job without high work stress, paid job with high work stress). High work stress is defined as ≥32 working hours per week combined with having high job strain.[7] Job strain was measured by the validated Dutch version of the Job Content Questionnaire (JCQ).[8] Missing values of maternal weight and height were imputed by means of a random imputation method using linear regression,[9] accounting for the differences among the ethnic groups.

Statistical analysis

Differences in the distribution of constitutional and environmental characteristics between the Dutch and ethnic minority groups and between generations were tested with either the Chi-square test for categorical variables or ANOVA with Bonferroni correction for continuous variables. Descriptive statistics were used to obtain the mean birth weights, birth weight percentiles and mean gestational ages per ethnic group.

Linear regression analyses were performed to estimate the size of differences in birth weight, standardized for GA, among ethnic groups, adjusting for above-mentioned determinants of birth weight. All covariates, except for maternal height, were treated as categorical variables. Analysis followed a predefined hierarchical format. Firstly, univariate analyses were performed to obtain the associations of ethnicity and all determinants separately with birth weight for GA. Secondly, two multiple linear regression models (forced entry method) were explored. The first model examined the association of ethnicity with birth weight for GA, adjusted for the constitutional determinants. The second model additionally adjusted for the environmental determinants. Data were analyzed using SPSS version 14.0 (SPSS Inc., Chicago, IL, USA). In all analyses a *P*-value < 0.05 was considered significant.

Results

Large differences in the prevalence of constitutional and environmental determinants were observed between Dutch and all ethnic minority groups (**Table 5.1**). Compared to the ethnic minority women, Dutch pregnant women were older, taller, higher educated, less often

depressed, had a lower BMI, and had more often a paid job in the first trimester of pregnancy. Dutch women were more frequently expecting their first child than the first generation ethnic minority women. Large differences were found for cohabitation status: while 50–64% of the Surinamese, Antillean, and Ghanaian women lived together with a partner, about 90% of the other women did. Smoking in the first trimester of pregnancy was most common among second generation Turkish women, while alcohol consumption was most common among Dutch women. Second generation ethnic groups were on average younger, taller, higher educated, and more frequently expecting their first child compared to the first generation women.

Mean birth weight ranged from 3223 g (second generation Surinamese newborns) to 3548 g (Dutch newborns) (**Table 5.2**). Birth weight distributions of all ethnic minority groups were shifted to the left compared to the Dutch birth weight distribution. Comparisons with the Bonferroni test showed a significantly lower mean birth weight for first and second generation Surinamese and Ghanaian newborns, and for first generation Antillean and other non-Dutch newborns. The Surinamese groups showed a significantly lower mean gestational age than the Dutch group. Within ethnic groups, no significant differences were found between the mean birth weights and gestational ages of infants born to first and second generation women.

Univariate linear regression analysis showed a significant association between all determinants and birth weight for GA (**Table 5.3**). All ethnic minority groups had lower mean birth weights for GA compared to the Dutch group, although not all group differences were significant. After adjustment for constitutional determinants (Model 1), ethnic differences in birth weight decreased substantially for all groups. Further adjustment for environmental determinants (Model 2) provided a little additional decrease or for some groups an increase in the ethnic differences in birth weight. The adjusted birth weights of Surinamese newborns remained lower than the Dutch birth weights, with a significant difference in weight of 98 g for the first generation and 159 g for the second generation ($P < 0.001$). Also Ghanaian and first generation Antillean women had significantly smaller newborns after adjustment (a difference of 121 g ($P = 0.001$) and 102 g ($P = 0.037$) respectively).

Table 5.1 Characteristics of the pregnant women, separated for first and second generation ethnic minority groups (n = 7118)

	Ethnicity – First generation							Ethnicity – Second generation				
	Dutch	Surinamese	Antillean	Turkish	Moroccan	Ghanaian	Other	Surinamese	Antillean	Turkish	Moroccan	Other
Number of women	3859	363	76	289	510	137	1187	180	28	72	119	298
Constitutional determinants												
Boy, %	49.9	52.6	48.7	49.8	53.7	46.7	49.6	51.7	39.3	51.4	47.1	48.7
Nulliparity, %	59.0	38.6*	64.5	37.0*	39.6*	33.6*	54.1*	65.6§	75.0	73.6*§	65.5§	60.1
Maternal age, %												
≤24 year	5.6	17.9*	31.6*	40.5*	25.9*	13.1	12.2*	53.9*§	25.0*	63.9*§	53.8*§	9.4
25–34 year	66.9	56.2	51.3	48.1	60.2	58.4	64.1	38.9	60.7	36.1	46.2	63.1
≥35 year	27.5	25.9	17.1	11.4	13.9	28.5	23.7	7.2	14.3	0.0	0.0	27.5
Maternal height (cm), mean (SD)	171 (6)	164 (6)*	168 (6)*	162 (5)*	164 (6)*	164 (7)*	165 (7)*	167 (6)*§	169 (6)	164 (6)*	166 (6)*§	169 (7)*§
Environmental determinants												
Education, %												
≤5 year	8.8	43.0*	35.5*	62.6*	60.2*	55.5*	28.3*	29.4*	17.9	43.1*§	30.3*§	12.1§
6–10 year	35.8	46.0	31.6	31.5	34.5	39.4	41.4	52.8	42.9	50.0	60.5	36.2
≥11 year	55.4	11.0	32.9	5.9	5.3	5.1	30.2	17.8	39.3	6.9	9.2	51.7
Living together with partner, %	90.8	57.3*	51.3*	95.5*	93.5*	53.3*	89.3	50.6*	64.3*	94.4	84.0*§	87.6
Maternal BMI, %												
Underweight	2.3*	4.7*	5.3*	2.4*	1.8*	0.0*	5.4*	6.7*§	7.1	4.2*	4.2*	3.4§
Normal weight	81.0	55.9	64.5	65.7	52.0	45.3	73.2	70.0	71.4	56.9	56.3	84.6
Overweight	13.0	24.5	18.4	21.5	33.3	35.8	16.8	16.1	14.3	26.4	33.6	8.7
Obesity	3.7	14.9	11.8	10.4	12.9	19.0	4.6	7.2	7.1	12.5	5.9	3.4

(continued)

Table 5.1 (continued)

	Ethnicity – First generation							**Ethnicity – Second generation**				
	Dutch	Surinamese	Antillean	Turkish	Moroccan	Ghanaian	Other	Surinamese	Antillean	Turkish	Moroccan	Other
Smoking, %												
None	89.8	87.6	97.4	82.7	97.5*	98.5	95.4	84.4	85.7	68.1*	96.6	85.9
<1 cigarette/day	2.9	4.4	0.0	4.8	0.4	0.0	0.8	3.9	7.1	5.6	0.0	4.4
1–5 cigarettes/day	3.9	3.6	1.3	6.6	1.2	1.5	1.6	8.9	3.6	19.4	0.8	5.0
>5 cigarettes/day	3.3	4.4	1.3	5.9	1.0	0.0	2.2	2.8	3.6	6.9	2.5	4.7
Alcohol consumption, %	29.5	12.1*	11.8*	0.7*	0.2*	10.2*	17.9*	8.9*	14.3	1.4*	0.8*	30.5
Depressed, %	6.9	20.9*	21.1*	21.8*	15.7*	10.2	13.8*	16.1*	17.9*	27.8*	27.7*§	9.1§
Work stress, %												
No paid job	17.5	46.8*	42.1*	78.2*	75.9*	69.3*	52.5*	52.8*	35.7*	58.3*§	57.1*§	17.4§
Low	76.7	48.5	56.6	19.0	22.0	25.5	43.8	40.0	64.3	33.3	38.7	78.2
High	5.8	4.7	1.3	2.8	2.2	5.1	3.7	7.2	0.0	8.3	4.2	4.4

** Significantly different from the Dutch group ($P < 0.05$).*

§ Significantly different from the first generation ($P < 0.05$).

Table 5.2 Birth weight distribution and gestational age per ethnic group, separated for first and second generation ethnic minority groups

		Birth weight distribution (in grams)			Gestational age (in weeks)
		Mean (SD)	25th percentile	75th percentile	Mean (SD)
Dutch		3548 (484)	3220	3850	39.7 (1.2)
Surinamese	1st generation	3311 (489)*	2990	3600	39.2 (1.2)*
	2nd generation	3223 (493)*	2865	3480	39.3 (1.3)*
Antillean	1st generation	3351 (511)*	3155	3658	39.5 (1.3)
	2nd generation	3475 (501)	3098	3834	39.5 (1.2)
Turkish	1st generation	3469 (463)	3150	3780	39.6 (1.3)
	2nd generation	3429 (433)	3129	3780	39.5 (1.2)
Moroccan	1st generation	3527 (482)	3182	3879	39.8 (1.3)
	2nd generation	3419 (455)	3142	3695	39.7 (1.3)
Ghanaian	1st + 2nd generation	3366 (454)*	3050	3658	39.4 (1.2)
Other	1st generation	3466 (469)*	3140	3780	39.7 (1.3)
	2nd generation	3511 (474)	3195	3773	39.7 (1.3)

** Significantly different from Dutch group (P < 0.05).*

Discussion

In this study term birth weight distributions of all ethnic minority groups were shifted to lower birth weights compared to the indigenous distribution. Constitutional determinants largely explained these ethnic disparities, while environmental determinants provided only a slightly additional explanation. An unexplained difference in birth weight persisted for black, i.e., Surinamese, Ghanaian, and (first generation) Antillean newborns, compared to Dutch newborns.

Previous studies in the Netherlands found similar patterns of crude birth weights in Dutch and ethnic minority groups, with the birth weight distributions of minority groups shifted to the left.[10-13] Also in other countries, like the USA and UK, large and unexplainable differences in the birth weights of newborns with a different ethnic background (white vs. black) have been observed.[14,15] Most studies, however, presented birth weights over the whole gestational age spectrum, leaving room for ambiguity by preterm birth. The Wilcox-Russell approach in our view is a valid tool to prevent such ambiguities.

Our distinction between constitutional and environmental determinants has few precedents. This distinction, in our view, is justified given the different consequences of lower birth weight depending on the underlying mechanism; if the normal birth weight distribution is shifted to lower birth weights without affecting mortality risk, preventive actions are less relevant. Furthermore, the general claim for ethnic disparities should be specified to those groups really in need for action (here the black group). The distinction between constitutional

Table 5.3 Results of the hierarchical linear regression analyses: differences in birth weight (in grams) – standardized for gestational age – between the ethnic groups, adjusted for constitutional and environmental determinants

		Univariate[a]		Model 1[b]		Model 2[c]	
		B	95% CI	*B*	95% CI	*B*	95% CI
Ethnicity							
Dutch (reference)		0.0		0.0		0.0	
Surinamese	1st generation	−161.7	−209.6, −113.9	−94.3	−141.6, −47.1	−98.3	−146.8, −49.7
	2nd generation	−255.3	−321.6, −189.0	−168.5	−233.7, −103.2	−159.3	−224.8, −93.9
Antillean	1st generation	−152.4	−253.0, −51.9	−82.1	−178.3, 14.1	−102.0	−197.6, −6.3
	2nd generation	−40.6	−205.2, 124.1	32.4	−124.4, 189.2	36.9	−117.7, 191.4
Turkish	1st generation	−54.0	−106.9, −1.0	42.3	−11.5, 96.1	36.6	−18.6, 91.7
	2nd generation	−82.2	−185.5, 21.1	62.7	−38.0, 163.2	68.0	−31.9, 167.9
Moroccan	1st generation	−21.4	−62.3, 19.5	49.0	7.5, 90.4	6.2	−38.2, 50.6
	2nd generation	−119.8	−200.6, −39.0	−24.3	−103.0, 54.5	−53.6	−132.0, 24.9
Ghanaian	1st + 2nd generation	−133.5	−209.0, −57.9	−74.6	−147.4, −1.7	−120.7	−195.1, −46.3
Other	1st generation	−72.1	−100.9, −43.3	8.6	−20.6, 37.8	5.2	−24.9, 35.4
	2nd generation	−33.6	−85.8, 18.6	5.3	−44.5, 55.1	18.9	−30.2, 67.9
Constitutional determinants							
Fetal sex							
Boy		0.0		0.0		0.0	
Girl		−134.9	−155.4, −114.4	−135.5	−155.1, −115.9	−136.4	−155.7, −117.1
Parity							
0		0.0		0.0		0.0	
≥1		156.7	136.2, 177.3	170.7	149.8, 191.5	160.7	139.6, 181.9
Age							
≤24 year		−110.6	−141.6, −79.6	−35.0	−67.0, −2.9	−11.9	−45.2, 21.3
25–34 year		0.0		0.0		0.0	
≥35 year		27.8	2.9, 52.6	−14.7	−38.8, 9.4	−17.3	−41.1, 6.6
Maternal height (cm)							
Linear		12.7	11.3, 14.1	13.6	12.1, 15.1	14.3	12.7, 15.8
Environmental determinants							
Education							
≤5 year		0.0				0.0	
6–10 year		21.7	−5.9, 49.3			2.3	−26.1, 30.7
≥11 year		77.3	49.8, 104.7			19.2	−13.2, 51.5
Living together with partner							
Yes		0.0				0.0	
No		−98.2	−128.7, −67.7			−21.3	−52.7, 10.2

(continued)

Table 5.3 (continued)

	Univariate[a]		Model 1[b]		Model 2[c]	
	B	95% CI	*B*	95% CI	*B*	95% CI
Maternal BMI						
Underweight	−189.8	−249.2, −130.3			−169.5	−225.5, −113.5
Normal weight	0.0				0.0	
Overweight	71.0	43.3, 98.8			90.1	63.2, 117.0
Obesity	116.8	72.8, 160.7			163.0	120.7, 205.3
Smoking						
No cigarettes	0.0				0.0	
<1 cigarettes/day	−135.0	−200.8, −69.3			−117.4	−179.7, −55.2
1–5 cigarettes/day	−145.1	−200.2, −90.0			−125.4	−177.8, −73.1
>5 cigarettes/day	−215.6	−275.1, −156.1			−205.6	−262.7, −148.6
Alcohol consumption						
No	0.0				0.0	
Yes	27.7	2.5, 52.9			−8.7	−33.6, 16.1
Depression						
No	0.0				0.0	
Yes	−53.1	−86.1, −20.2			−25.0	−56.6, 6.7
Work stress						
No paid job	−28.3	−50.4, −6.3			8.3	−16.1, 32.6
Low	0.0				0.0	
High	−116.8	−165.5, −68.2			−82.6	−128.0, −37.1

[a] *Univariate analysis (B: unstandardized regression coefficient; 95% confidence interval).*

[b] *Model 1: Multiple regression, adjusted for constitutional determinants.*

[c] *Model 2: Multiple regression, adjusted for constitutional and environmental determinants.*

and environmental determinants can be debated. In contrast to other studies[5] we did not include prepregnancy maternal weight as a constitutional determinant of fetal growth, because the size of the fetus is determined more by maternal height than by maternal weight.[16] Only weights at the extremes (i.e., under- and overweight for height) influence fetal growth, resulting in either pathologically restricted or enlarged newborns.[17]

At first sight, the birth weight distribution of all ethnic minority groups was shifted to lower birth weights compared to the Dutch group. After standardization for GA and adjustment for constitutional determinants of fetal growth, Turkish, Moroccan and other non-Dutch newborns appeared to have a comparable size at birth as Dutch newborns. This suggests a limited constitutional growth potential rather than a pathological growth restriction for the majority of newborns in these groups. Maternal height was by far the most important determinant of birth weight at term ($R^2 = 0.048$; followed by parity: $R^2 = 0.022$), stressing the

dominance of constitutional rather than environmental determinants in ethnic differences in birth weight. This minimal role of environmental determinants interferes with the commonly held preventive view that much can be gained here. Smoking, maternal BMI and high work-related stress are significant and modifiable environmental determinants of fetal growth on the individual level, certainly in need for preventive actions, but their potential to reduce the ethnic disparities in birth weight is low.

Although constitutional rather than environmental determinants were also responsible for a large shift in the birth weight distribution of the Surinamese, Ghanaian and Antillean group, these newborns remained substantially smaller than the Dutch newborns. Additional explanation by other environmental factors, like diabetic[18] and hypertensive disorders,[19] or nutritional habits[20,21] (e.g., folic acid, fatty acids) must be investigated. The role of nutrition in this cohort is currently being examined in detailed analysis. Another explanation could probably be find in the arising field of cumulative risk.[22] A cumulative exposure to multiple risk factors during pregnancy may substantially increase the risk of a lower birth weight at term, especially among the vulnerable ethnic minority groups. Instead of such additional pathological explanations of the lower birth weights among black newborns, a broader constitutional explanation (including genetics) might also be appropriate.[23] Lower population birth weights at term are not necessarily related to higher perinatal morbidity and mortality,[24-26] suggesting some genetic influences. Whether the majority of black newborns is pathologically growth restricted or constitutionally small is not yet clear and needs further investigation.

We expected the ethnic disparities in birth weight to decrease across generations as a result of the higher educational attainment of the second generation, however, we found the opposite for most groups. Previous studies in the UK and the USA also reported lower or similar birth weights in second generation ethnic minority groups compared with the first generation.[27-30] This lack of an intergenerational increase in birth weight at term supports the minimal role of environmental determinants. That both first and second Surinamese newborns were substantially smaller than the Dutch newborns after adjustment can either be the result of genetic influences[30,31] and/or the result of unspecified sociocultural determinants shared by the different generations.[30] Because the last explanation requires the identification of new, strong, and independent environmental risk factors,[4,33] a genetic role seems more likely to us. More evidence for a genetic component in fetal growth should be explored by comparing the birth weights of third and next generation ethnic groups, or by exploring DNA markers using admixture mapping.[34]

Our study population consisted of a large, unselected cohort of pregnant women, which adequately represented the main ethnic groups in Amsterdam.[35] Although there was selective participation, with a higher participation rate among the Western groups, selection bias was absent.[36] The exclusion of women with incomplete data might also have led to a relative healthier sample for analysis. The small number of (second generation) Antillean women limited the power for detailed analysis, although the numbers were still larger than

comparable studies.[11,12] We could not split the Surinamese group into a Creole and Hindustani group as detailed information was available only for a sub sample of women. However, as we did not observe significant differences in birth weight between known Hindustani and Creole newborns (results not shown), we felt it appropriate to include both in the Surinamese group.

In conclusion, the birth weight differences between Dutch newborns and Turkish, Moroccan, and other non-Dutch newborns were largely explained by constitutional determinants, limiting the need for prevention. The substantially lower birth weights of black newborns, however, remains to be elucidated. Future research should focus on the underlying mechanism of this disparity, by searching for unexplored environmental risk factors or for cumulative risk effects, and by investigating the weight-related population mortality rates. Within the ABCD study, future research will focus on whether constitutional and environmental risk factors explain the ethnic differences in preterm birth. Considering the small influence of the investigated modifiable environmental factors, the potential to reduce ethnic disparities in term birth weight on a population level seems limited. Nevertheless, preventive actions on in particular smoking, maternal BMI, and working-related stress remain important to improve the individual health of newborns.

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References

1. Wilcox AJ. On the importance - and the unimportance - of birthweight. Int J Epidemiol 2001;30:1233–41.
2. Adams M, Andersen AM, Andersen PK, et al. Sostrup statement on low birthweight. Int J Epidemiol 2003;32:884–5.
3. Evans S, Alberman E, Pashley J, Hampton B. International Collaborative Effort (ICE) on birthweight; plurality; and perinatal and infant mortality. II: Comparisons between birthweight distributions of births in member countries from 1970 to 1984. Acta Obstet Gynecol Scand 1989;68:11–7.
4. Goldenberg RL, Cliver SP, Mulvihill FX, et al. Medical, psychosocial, and behavioral risk factors do not explain the increased risk for low birth weight among black women. Am J Obstet Gynecol 1996;175:1317–24.
5. Mamelle N, Cochet V, Claris O. Definition of fetal growth restriction according to constitutional growth potential. Biol Neonate 2001;80:277–85.
6. Radloff LS. The CES-D scale: a self-reported depression scale for research in the general population. Applied Psychological Measurement 1977;1:385–401.
7. Vrijkotte TG. Working condition and birth weight in a multi-cultural cohort: the ABCD-study. Eur J Epidemiol 2006;21(suppl 1):38 (abstr).
8. Karasek R, Brisson C, Kawakami N, Houtman I, Bongers P, Amick B. The Job Content Questionnaire (JCQ): an instrument for internationally comparative assessments of psychosocial job characteristics. J Occup Health Psychol 1998;3:322–55.
9. Allison PD. Multiple imputation: basics. In: Allison PD. Missing data. Thousand Oaks, CA: Sage Publications, 2001:27–41.
10. Doornbos JP, Nordbeck HJ, van Enk AE, Muller AS, Treffers PE. Differential birthweights and the clinical relevance of birthweight standards in a multiethnic society. Int J Gynaecol Obstet 1991;34:319–24.
11. Verkerk PH, Zaadstra BM, Reerink JD, Herngreen WP, Verloove-Vanhorick SP. Social class, ethnicity and other risk factors for small for gestational age and preterm delivery in The Netherlands. Eur J Obstet Gynecol Reprod Biol 1994;53:129–34.
12. Drooger JC, Troe JW, Borsboom GJ, et al. Ethnic differences in prenatal growth and the association with maternal and fetal characteristics. Ultrasound Obstet Gynecol 2005;26:115–22.
13. van der Wal MF, Uitenbroek DG, van Buuren S. Geboortegewicht van Amsterdamse kinderen naar etnische afkomst (Birthweight of children in Amsterdam by ethnic origin). TSG Tijdschrift voor Gezondheidswetenschappen 2000;78:15–20 (in Dutch).
14. Wilcox M, Gardosi J, Mongelli M, Ray C, Johnson I. Birth weight from pregnancies dated by ultrasonography in a multicultural British population. BMJ 1993;307:588–91.
15. Shiono PH, Rauh VA, Park M, Lederman SA, Zuskar D. Ethnic differences in birthweight: the role of lifestyle and other factors. Am J Public Health 1997;87:787–93.
16. Blair EM, Liu Y, de Klerk NH, Lawrence DM. Optimal fetal growth for the Caucasian singleton and assessment of appropriateness of fetal growth: an analysis of a total population perinatal database. BMC Pediatr 2005;5:13.
17. Catalano PM, Ehrenberg HM. The short- and long-term implications of maternal obesity on the mother and her offspring. BJOG 2006;113:1126–33.

18. Rosenberg TJ, Garbers S, Lipkind H, Chiasson MA. Maternal obesity and diabetes as risk factors for adverse pregnancy outcomes: differences among 4 racial/ethnic groups. Am J Public Health 2005;95:1545–51.
19. Fang J, Madhavan S, Alderman MH. The influence of maternal hypertension on low birth weight: differences among ethnic populations. Ethn Dis 1999;9:369–76.
20. Lumey LH, Stein AD. Offspring birth weights after maternal intrauterine undernutrition: a comparison within sibships. Am J Epidemiol 1997;146:810–9.
21. Ramakrishnan U, Manjrekar R, Rivera J, Gonzáles-Cossío T, Martorell R. Micronutrients and pregnancy outcome: a review of the literature. Nutr Res 1999;19:103–59.
22. Burchinal MR, Roberts JE, Hooper S, Zeisel SA. Cumulative risk and early cognitive development: a comparison of statistical risk models. Dev Psychol 2000;36:793–807.
23. van den Oord EJ. Ethnic differences in birth weight: maternal effects emerge from an analysis involving mixed-race US couples. Ethn Dis 2006;16:706–11.
24. Vangen S, Stoltenberg C, Skjaerven R, Magnus P, Harris JR, Stray-Pedersen B. The heavier the better? Birthweight and perinatal mortality in different ethnic groups. Int J Epidemiol 2002; 31:654–60.
25. Wilcox AJ, Russell IT. Birthweight and perinatal mortality: II. On weight-specific mortality. Int J Epidemiol 1983;12:319–25.
26. Graafmans WC, Richardus JH, Borsboom GJ, et al. Birth weight and perinatal mortality: a comparison of "optimal" birth weight in seven Western European countries. Epidemiology 2002;13:569–74.
27. Harding S, Rosato MG, Cruickshank JK. Lack of change in birthweights of infants by generational status among Indian, Pakistani, Bangladeshi, Black Caribbean, and Black African mothers in a British cohort study. Int J Epidemiol 2004;33:1279–85.
28. Draper ES, Abrams KR, Clarke M. Fall in birth weight of third generation Asian infants. BMJ 1995;311:876.
29. Margetts BM, Mohd Yusof S, Al Dallal Z, Jackson AA. Persistence of lower birth weight in second generation South Asian babies born in the United Kingdom. J Epidemiol Community Health 2002;56:684–7.
30. David RJ, Collins RW. Differing birth weight among infants of U.S.-born blacks, African-born blacks, and U.S.-born whites. New Engl J Med 1997;337:1209–14.
31. Amante A, Borgiani P, Gimelfarb A, Gloria-Bottini F. Interethnic variability in birth weight and genetic background: a study of placental alkaline phosphatase. Am J Phys Anthropol 1996;101:449–53.
32. Dunger DB, Petry CJ, Ong KK. Genetic variations and normal fetal growth. Horm Res 2006;65(suppl 3):34–40.
33. Foster HW, Wu L, Bracken MB, Semenya K, Thomas J, Thomas J. Intergenerational effects of high socioeconomic status on low birthweight and preterm birth in African Americans. J Natl Med Assoc 2000;92:213–21.
34. Frank R. What to make of it? The (Re)emergence of a biological conceptualization of race in health disparities research. Soc Sci Med 2007;64:1977–83.
35. van Zee W, Hylkema C, eds. Kerncijfers Amsterdam 2004 (Key figures Amsterdam 2004). Amsterdam: Stadsdrukkerij Amsterdam, 2004 (in Dutch).
36. Tromp M, van Eijsden M, Ravelli AC, Bonsel GJ. Anonymous non-response analysis in the ABCD-cohort study enabled by probabilistic record linkage (submitted 2007).

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Chapter 6

Association between short interpregnancy intervals and term birth weight: the role of folate depletion

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Abstract

Background

Maternal folate depletion has been proposed as primary explanation for the excess risk of fetal growth restriction associated with short interpregnancy intervals.

Objective

To evaluate the folate depletion hypothesis in a community-based cohort of pregnant women.

Design

Using a subsample of the cohort (multiparous participants who delivered a singleton liveborn infant, n = 3153), we investigated the relationship between increasing interpregnancy interval (1–24 months, natural log transformation) and birth weight and small-for-gestational-age (SGA) risk in three strata of maternal periconceptional folic acid use: "nonuse", "late use" (start after conception), and "early use" (start before conception).

Results

Each unit increase in the interpregnancy interval on the natural log (ln) scale was associated with a birth weight increase of 63.1 g (SE 20.3, $P = 0.002$). This relationship was mitigated by folic acid use: the increase in birth weight per unit increase in ln(interpregnancy interval) was 165.2 (39.6) g for nonuse ($P < 0.001$), 33.5 (35.6) g for late use ($P = 0.347$), and −5.8 (33.6) g for early use ($P = 0.861$). The birth weight differences were directly translated into SGA risk. Odds ratios per unit increase in ln(interpregnancy interval) were significant for the group in total (0.61; 95% CI: 0.46, 0.82) and for nonuse (0.38; 95% CI: 0.24, 0.60), and nonsignificant for late use (0.83; 95% CI: 0.48, 1.44) and for early use (1.28; 95% CI: 0.58, 2.84).

Conclusion

Folate depletion apparently contributes to the excess risk of fetal growth restriction associated with short interpregnancy intervals. As a preventive option, postnatal supplementation may be beneficial, but confirmation is needed.

Introduction

Several studies have reported increased risks of the adverse pregnancy outcomes low birth weight (LBW) and small for gestational age (SGA) after short interpregnancy intervals.[1-13] Yet, a general explanation for these excess risks, in a recent meta-analysis estimated to be just over 60% for LBW and 25% for SGA,[14] is still lacking. Some authors have attributed the higher risk of poor pregnancy outcomes to factors associated with – rather than causally related to – short interpregnancy intervals, such as maternal sociodemographic characteristics and lifestyle.[15-17] However, accumulating evidence from the studies that did extensively control for such risk factors[3-5,7,10-12] suggests that the adverse outcomes are not merely the results of confounding.

A plausible, more causal, hypothesis that has been put forward to explain the excess risk is the nutritional depletion hypothesis,[18,19] which states that women with closely spaced births have insufficient time to restore the nutritional reserves needed to support fetal growth and development in the subsequent pregnancy. Folate depletion in particular has been proposed as the nutritional factor that contributes most to the risk of fetal growth restriction.[20] During pregnancy, folate is mobilized from maternal stores to meet the increasing demands of mother and child. If dietary supply is low, concentrations begin to decline from the fifth month of pregnancy onwards, and continue to decline until several weeks after delivery.[21,22] As repletion of stores may then take several months,[22] mothers conceiving a subsequent child within these first months postpartum are at increased risk of folate deficiency. As a consequence, their offspring may be at higher risk of intrauterine growth restriction and LBW, which have been associated with both low folate and high homocysteine concentrations (as marker for folate deficiency)[23] before.[24-29]

In the present study, we evaluate the folate depletion hypothesis as an explanation for short interval-associated adverse outcomes, by examining in different strata of folic acid supplement use the relationship of interpregnancy interval with infant birth weight and SGA risk at term. If the hypothesis is true, we expect to (a) observe an increased risk of fetal growth restriction (i.e., lower birth weight and higher SGA risk) at short intervals, which diminish with increasing interval length, and (b) find a mitigating influence of folic acid supplement use on these risks. More specifically, lower birth weight and higher SGA risk are particularly expected among nonusers, who are at highest risk of folate depletion, and not, or to a lesser extent among early (start before conception) or late (start after conception) users. If our findings corroborate the hypothesis, this could open new avenues for the prevention of adverse pregnancy outcomes among women conceiving their second or subsequent child, in particular those who become pregnant shortly after their previous pregnancy.

Subjects and methods

Study population and design

This study is part of the Amsterdam Born Children and their Development (ABCD) study, a prospective community-based cohort study that examines the relationship between maternal lifestyle and psychosocial conditions during pregnancy and the child's health at birth as well as in later life. The design of the study has been described to a large extent previously.[30] In short, between January 2003 and March 2004, pregnant women living in Amsterdam were invited to enroll in the ABCD study during their first antenatal visit to the obstetric care provider (around the 12th week of gestation), and requested to complete a questionnaire, covering sociodemographic data, obstetric history, lifestyle, dietary habits, and psychosocial factors. The questionnaire was available in Dutch as well as in English, Turkish, and Arabic for immigrant women. In total, 8266 of the 12 373 pregnant women invited to participate returned the pregnancy questionnaire (response rate 67%). Of this group, 7738 women gave birth to a viable singleton infant for whom information on birth weight and pregnancy duration was available. For the present study we excluded all women with preterm deliveries (n = 410) as well as all women with first-time deliveries (n = 3993). The total number available for analyses was 3335.

The study was approved by the Medical Ethical Committees of the participating hospitals and the Registration Committee of Amsterdam. All participating women gave written consent.

Measurements

Primary outcome variables for the present study were birth weight (continuous in grams) and SGA (yes, no) at term, with SGA defined as a birth weight below the 10th percentile for gestational age on the basis of sex- and parity-specific standards from the Netherlands Perinatal Registry (data available from the authors upon request). Data on date of delivery, infant sex, birth weight, and gestational age [ultrasound-based or, if unavailable (<10%), on the timing of the last menstrual period] as recorded by the obstetric care providers were obtained through the Youth Health Department at the Municipal Health Service in Amsterdam.

Interpregnancy interval was calculated as the number of months between date of delivery and date of preceding birth, diminished by pregnancy duration. The latter was defined by the gestational age at the time of delivery in days. The use of folic acid supplements was assessed by the question: "Have you taken folic acid, either as a single supplement or as part of a multivitamin supplement, before or during your pregnancy?". As the general recommendation is to start taking supplements at least four weeks before conception,[31] women were also asked to indicate when they had started taking supplements: (a) before conception or (b) after conception. Women who took folic acid supplements and had started before conception were classified as "early users" of folic acid. Those who took folic acid supplements but had

started after conception were classified as "late users", and those who did not use folic acid supplements at all as "nonusers".

A number of maternal physiologic, obstetric, lifestyle, and sociodemographic characteristics obtained via the questionnaire were considered covariables. Physiologic and obstetric variables included age (<25, 25–34, ≥35 years), parity (1, 2, ≥3), height (cm), pregnancy intention as measured by questioning whether the respondent had wanted to become pregnant (yes, no), and start of prenatal care (<18 weeks, 18–23, ≥24 weeks). Lifestyle variables included alcohol consumption before or during early pregnancy (self-reported previous week's behavior and behavioral change since pregnancy, recoded into: "no", "yes, but not since pregnancy", "yes, also during pregnancy"), smoking habits before and during early pregnancy (self-reported previous week's behavior and behavioral change since pregnancy, recoded into: "no", "yes, but not since pregnancy", "yes, also during pregnancy"), pregravid body mass index (BMI, kg/m^2) based on self-reported height and weight, and psychosocial stress (presence of 0, 1, or ≥2 stressors). A random imputation procedure using linear regression analysis was used to complete missing data on height (3.8% missing) or weight (9.9% missing).[32] Psychosocial stressors were measured by validated Dutch versions of internationally accepted questionnaires and included depression,[33] general anxiety,[34] pregnancy-related anxiety,[35] parenting stress,[36] and work stress.[37] Thresholds for nonnormal scores, assuming a representative group of pregnant women, were by design chosen at the 90th percentile. Lastly, sociodemographic factors were cohabitant status (living together with partner or living alone), educational attainment after primary school (<6, 6–10, ≥11 years), and country of birth (the Netherlands, Surinam, Turkey, Morocco, other non-Western country, other Western country).

Statistical analysis

Of the 3335 women included, 75 women did not provide information on interpregnancy interval (n = 55) or folic acid supplement use (n = 20), while 107 women had one or more missing values on the above-mentioned covariables (missing rate per item ≤2%). After exclusion of these respondents the final sample for analysis was 3153.

After a descriptive analysis of the infant and maternal characteristics, we performed univariate and multivariate regression analyses to estimate the association of interpregnancy interval as categorical variable with term birth weight (linear regression) and SGA (logistic regression). For interpregnancy interval, previously defined categories were used: 0–5, 6–11, 12–17, 18–23, 24–59, and ≥60 months.[14] Models were adjusted for above-mentioned maternal physiologic, obstetric, lifestyle, and sociodemographic characteristics (see *Measurements*). For birth weight as outcome variable, adjustments were also made for infant sex and gestational age. These analyses allowed us to evaluate the actual presence of an association in our population and relate our findings to previous studies.

To specifically test the folate depletion hypothesis, we performed predefined multivariate regression analysis including the natural log transformation of the interpregnancy interval

as continuous variable. First, multivariate models were fit to describe the relation between increasing length of interpregnancy interval (1 to 24 months) and birth weight (linear regression model) or SGA (logistic regression model). Although both short and long interpregnancy intervals have previously been associated with adverse outcomes, the latter association is not expected to be influenced by folate depletion;[14,20] therefore, long intervals were excluded for this part of the analysis. The particular cutoff of 24 months and natural log transformation were chosen because the risks associated with short intervals have been shown to normalize within this time frame, following an inverse J-shaped pattern (after an initial steep decline in risk, a gradual leveling off with increasing interval length).[3,6,9-14] Second, stratified analysis using the previously defined models for birth weight and SGA was performed to evaluate the mitigating influence of folic acid supplement use. Strata were no use, late use, and early use (see *Measurements*). The hypothesized mitigating influence of folic acid use was subsequently statistically tested by including both supplement use and interaction terms for supplement use and interpregnancy interval in the models (test for effect modification).

Associations were considered statistically significant at $P < 0.05$. All analyses were conducted in SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

Characteristics of the mother-infant pairs included (n = 3153) are presented in **Table 6.1**. Mean infant birth weight (± SD) was 3579 (± 497) g, mean gestational age at birth 40.0 (± 1.2) wk. In total, 12.5% of the infants were born SGA. Sixty-four percent of the mothers had used folic acid supplements during pregnancy; 29% had started taking these supplements after conception, and 35% had already started taking these supplements before conception.

Less than 4% of the women (n = 124) became pregnant within 6 months after the previous delivery, whereas 16% of the women (n = 513) had intervals of 5 years or longer (**Table 6.2**). In univariate analyses, both short (<6 months) and long (24–59, ≥60 months) interval categories reduced birth weight, with a corresponding increase in SGA risk for intervals <6 months and ≥60 months. After adjustment for relevant maternal and infant characteristics, these associations persisted for the short interval category (mean estimated difference in birth weight −142.1 g, SE 44.1; odds ratio (OR) for SGA 2.12, 95% CI 1.23, 3.65; reference 18–23 months), but not for the 24–59 month interval category. Intervals exceeding 60 months were no longer associated with SGA risk, but remained negatively associated with birth weight (−66.6 g, SE 30.8; reference 18–23 months).

Table 6.1 Infant and maternal characteristics (n = 3153)

	Frequency or value
Infant characteristics	
Infant sex (% boy)	50.0
Birth weight (g)	3579 ± 497[a]
Gestational age at birth (wk)	40.0 ± 1.2
Small-for-gestational-age births (%)	12.5
Maternal characteristics[b]	
Folic acid supplement use (%)	
No	36.0
Yes, started after conception (late use)	29.4
Yes, started before conception (early use)	34.6
Age (%)	
<25 years	7.1
25–34 years	59.6
≥35 years	33.3
Parity (%)	
1	71.5
2	20.1
≥3	8.4
Height (cm)	167.7 ± 7.4
Unintended pregnancy (%)	9.3
Start prenatal care (%)	
<18 weeks	89.0
18–23 weeks	7.6
≥24 weeks	3.4
Alcohol consumption (%)	
No	47.1
Yes, but not since pregnancy	30.8
Yes, also during pregnancy	22.1
Smoking (%)	
No	80.9
Yes, but not since pregnancy	10.0
Yes, also during pregnancy	9.1
Pregravid BMI (kg/m^2)	23.7 ± 4.3
Psychosocial stressors (%)	
0	71.3
1	17.0
≥2	11.7

(continued)

Table 6.1 (continued)

	Frequency or value
Cohabitant status (% living alone)	11.5
Education (%)	
0–5 years	27.9
6–10 years	36.8
≥11 years	35.4
Country of birth (%)	
The Netherlands	57.4
Surinam	6.9
Turkey	5.6
Morocco	9.3
Other non-Western country	13.8
Other Western country	7.0

[a] *Mean ± SD (all such values).*

[b] *As determined in pregnancy; median (interquartile range) for pregnancy duration at questionnaire completion: 16 (14–19) weeks.*

The results of the regression analyses describing the relationship of increasing interpregnancy interval (1–24 months) with birth weight and SGA in our cohort are presented in **Figure 6.1**. Each unit increase in the interpregnancy interval expressed on a natural log scale was associated with an increase in birth weight of 63.1 g (SE 20.3, $P = 0.002$), and, correspondingly, a decrease in SGA risk of approximately 40% (OR: 0.61, 95% CI: 0.46, 0.82). In order to illustrate this relation on the original scale, we calculated the mean birth weight differences and ORs for SGA using month 1 as reference. These differences were 112.9 (95% CI: 41.5, 184.2) g at 6 months, 156.4 (57.5, 255.3) g at 12 months, 182.2 (67.0, 297.5) g at 18 months, and 200.5 (73.8, 327.3) g at 24 months. Estimated ORs for SGA were 0.42 (95% CI: 0.24, 0.70) at 6 months, 0.30 (0.15, 0.61) at 12 months, 0.24 (0.11, 0.56) at 18 months, and 0.21 (0.08, 0.53) at 24 months. Stated in reverse, counting down from month 24 as the presumed optimal interpregnancy interval, birth weight differences and 95% confidence intervals were −18.3 (−29.8, −6.7) g, −44.1 (−72.0, −16.2) g, −87.7 (−143.1, −32.2) g, and −200.5 (−327.3, −73.8) g at month 18, 12, 6, and 1 respectively, with corresponding ORs and 95% confidence intervals for SGA of 1.15 (1.05, 1.25), 1.41 (1.15, 1.72), 1.97 (1.33, 2.95), and 4.74 (1.89, 11.84).

Table 6.2 Results of the regression analyses relating birth weight and SGA to interpregnancy interval as defined in categories (n = 3153)[a]

Interpregnancy interval (months)	<6	6–11	12–17	18–23	24–59	≥60
n	124	487	510	407	1112	513
Birth weight (g)[b]	3461 ± 495	3610 ± 480	3617 ± 462	3658 ± 508	3577 ± 501	3482 ± 512
B_{crude} ± SE[c]	−197.1 ± 50.8*	−48.3 ± 33.2	−40.8 ± 32.8	0.0	−80.8 ± 28.6*	−176.5 ± 32.8*
B_{adj} ± SE[d]	−142.1 ± 44.1*	−6.0 ± 28.9	−14.0 ± 28.4	0.0	−22.2 ± 25.2	−66.6 ± 30.8*
SGA (%)	22.8	10.5	8.4	11.5	12.1	17.5
OR_{crude} (95% CI)[e]	2.26 (1.34, 3.78)*	0.90 (0.59, 1.36)	0.71 (0.46, 1.09)	1.00	1.06 (0.74, 1.51)	1.63 (1.12, 2.38)*
OR_{adj} (95% CI)[f]	2.12 (1.23, 3.65)*	0.88 (0.57, 1.36)	0.69 (0.44, 1.08)	1.00	0.85 (0.59, 1.24)	1.06 (0.69, 1.63)

[a] *Linear regression analysis for birth weight as dependent variable and interpregnancy interval in categories as primary independent variable; logistic regression analysis for SGA as dependent variable and interpregnancy interval in categories as primary independent variable. SGA, small for gestational age (birth weight < 10th percentile for gestational age based on sex- and parity-specific standards).*

[b] *Mean ± SD.*

[c] *B_{crude} ± SE: crude regression coefficient ± standard error.*

[d] *B_{adj} ± SE: regression coefficient ± standard error adjusted for gestational age at birth (linear and quadratic), infant sex, maternal age, height, parity, maternal pregravid BMI (linear and quadratic), smoking before and during pregnancy, alcohol consumption before and during pregnancy, psychosocial stress, pregnancy intention, cohabitant status, education, and ethnicity.*

[e] *OR_{crude} (95% CI): odds ratio (95% confidence interval).*

[f] *OR_{adj} (95% CI): odds ratio (95% confidence interval) adjusted for maternal age, height, maternal pregravid BMI (linear and quadratic), smoking before and during pregnancy, alcohol consumption before and during pregnancy, psychosocial stress, pregnancy intention, cohabitant status, education, and ethnicity. (Note: SGA is already adjusted for gestational age, infant sex, and parity).*

** P < 0.05 for comparison with reference category (18–23 months).*

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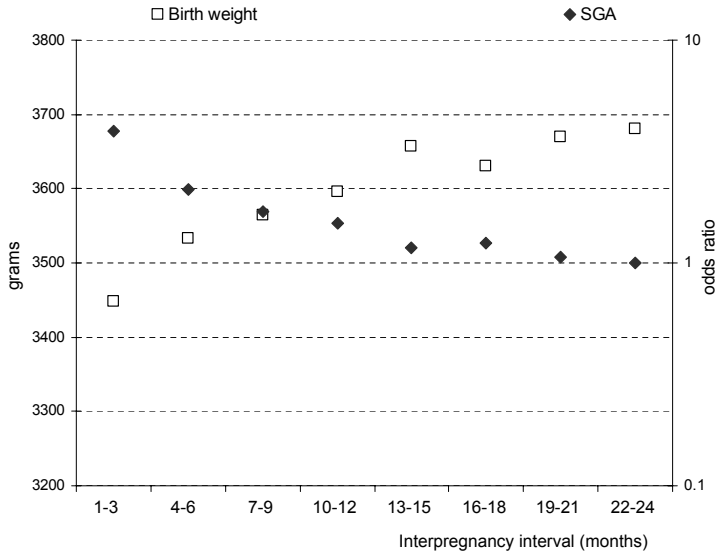

Figure 6.1 Estimated mean birth weight (linear regression) and odds ratios for small for gestational age (SGA) (logistic regression) in the ABCD cohort as a function of interpregnancy interval.
Estimates for birth weight were derived from a continuous multivariate linear regression model with birth weight as dependent and ln(interpregnancy interval) as primary independent variable; odds ratios for SGA were derived from a multivariate logistic regression model with SGA as dependent and ln(interpregnancy interval) as primary independent variable. In both models, adjustments were made for a predefined set of covariables (see footnote Table 6.2). For presentation purposes monthly intervals were combined in three-month categories, n = 43, 127, 244, 301, 263, 224, 204, and 180 at consecutive interval categories (n for birth weight analysis similar to n for SGA analysis).

In line with the hypothesis, we observed a mitigating effect of folic acid supplement use on the relationship of interpregnancy interval with birth weight (**Figure 6.2**) and SGA (**Figure 6.3**). Stratified analysis showed that in both early and late supplement users the association between interval and birth weight or SGA no longer existed; the change in birth weight per unit increase in ln(interpregnancy interval) was −5.9 g (SE 33.6) for early users and 33.5 g (35.6) for late users; the corresponding OR for SGA was 1.28 (95% CI: 0.58, 2.84) for early users and 0.83 (95% CI: 0.48, 1.44) for late users respectively. In contrast, for nonusers we observed a significant birth weight increase of 165.2 g (SE 39.6, $P < 0.001$) per unit increase in ln(interpregnancy interval), with a corresponding decrease in SGA risk of 60% (OR: 0.38, 95% CI: 0.24, 0.60). Consequently, mean birth weight differences in the nonuse group, taking the optimal interpregnancy interval of 24 months as reference, were −47.9 (95% CI: −70.4, −25.4) g at 18 months, −115.6 (−170.0, −61.3) g at 12 months, −229.6 (−337.5, −121.7) g at 6 months, and −525.3 g (−772.1, −278.5) at 1 month; corresponding ORs for SGA were 1.33 (95% CI: 1.16, 1.52), 1.98 (1.43, 2.75), 3.89 (2.02, 7.48), and 22.35 (5.01, 99.76) respectively. The interaction model confirmed that the effect modification as observed in the stratified analysis was significant for both birth weight ($P = 0.001$) and SGA ($P = 0.008$).

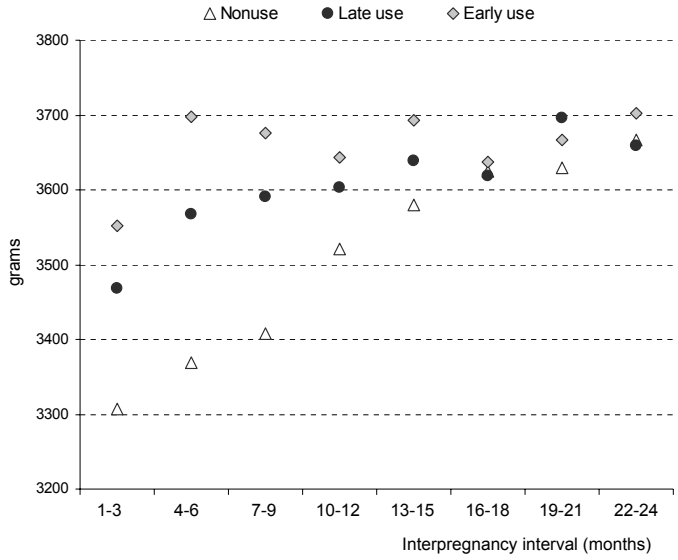

Figure 6.2 Estimated mean birth weight (linear regression) in the ABCD cohort as a function of interpregnancy interval in three strata of folic acid supplement use.

Estimates for birth weight were derived from a multivariate linear regression model with birth weight as dependent and ln(interpregnancy interval) as primary independent variable. Adjustments were made for a predefined set of covariables (see footnote Table 6.2). Analyses were stratified for folic acid supplement use: nonuse (n = 414), late use (n = 537), and early use (n = 635). For presentation purposes monthly intervals were combined in three-month categories. Nonuse: n = 21, 37, 69, 77, 60, 53, 47, and 50 at consecutive interval categories; late use: n = 13, 48, 97, 111, 80, 63, 78, and 47 at consecutive interval categories; early use: n = 9, 42, 78, 113, 123, 108, 79, and 83 at consecutive interval categories.

Discussion

The results of this large, prospective cohort study not only confirm previous research showing a negative association between short interpregnancy intervals and fetal growth,[1-14] but moreover support the explanatory hypothesis of folate depletion.[20] Among those most at risk of folate depletion – the nonusers of folic acid supplements – we observed an increased risk of fetal growth restriction (as reflected by a lower mean birth weight and higher SGA risk) at short interpregnancy intervals, which diminished with increasing interval length. In contrast, no significant interval-associated decrease in birth weight or increase in SGA risk was observed among supplement users. These observations imply that the adverse effects of interpregnancy intervals shorter than 6 months could be preventable by the use of folic acid supplements in the period between the consecutive pregnancies.

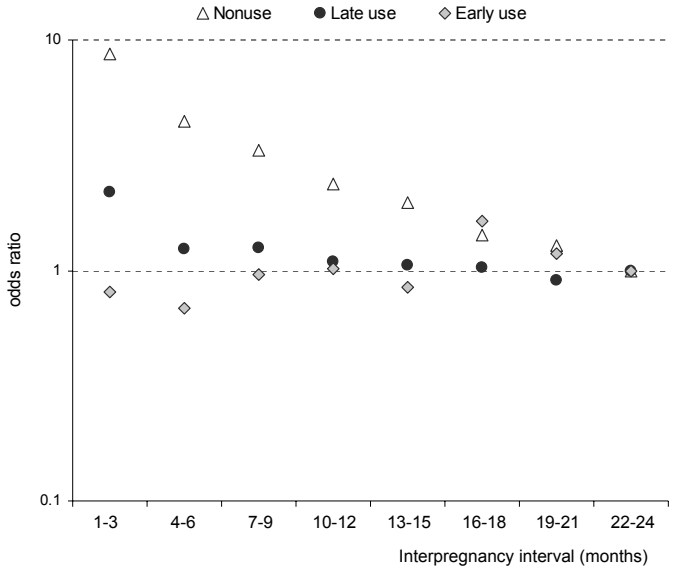

Figure 6.3 Estimated odds ratios for small for gestational age (SGA) (logistic regression) in the ABCD cohort as a function of interpregnancy interval in three strata of folic acid supplement use.

Odds ratios for SGA were derived from a multivariate logistic regression model with SGA as dependent and ln(interpregnancy interval) as primary independent variable. Adjustments were made for a predefined set of covariables (see footnote Table 6.2). Analyses were stratified for folic acid supplement use: nonuse (n = 414), late use (n = 537), and early use (n = 635). For presentation purposes monthly intervals were combined in three-month categories. Nonuse: n = 21, 37, 69, 77, 60, 53, 47, and 50 at consecutive interval categories; late use: n = 13, 48, 97, 111, 80, 63, 78, and 47 at consecutive interval categories; early use: n = 9, 42, 78, 113, 123, 108, 79, and 83 at consecutive interval categories.

Following the hypothesis, the mitigating influence of folic acid supplementation was expected to be dose-dependent. Indeed, we observed the interval-associated risks of lower birth weight and SGA among late users (i.e., start after conception) to be intermediate between the interval-associated risks among nonusers and early users (i.e., start before conception). The biological plausibility of this dose-dependency lends further credibility to a true causal role of folate depletion in the association between interpregnancy interval and adverse pregnancy outcomes. Folate has a fundamental role in the process of cell division because of its role in DNA synthesis. Particularly during pregnancy – a time of tissue growth and sustained cell division – the need for folate increases.[38] Where low maternal folate concentrations may negatively influence fetal growth and development, folate supplementation to restore folate status may counterbalance these effects. The largest reduction in risk may thus be expected from supplementation starting prior to conception, ensuring adequate folate status during the critical stage of embryonic development.[20] Indeed, previous studies have shown a preventive effect of higher folate intakes on adverse pregnancy outcomes particularly in women at high risk of folate deficiency.[39,40]

Given the unavoidable nonexperimental setting of our study, alternative explanations for our observations should be considered. We cannot exclude the possibility that our results are explained by maternal characteristics associated with supplement use (e.g., being more health-conscious), or more specifically by the concurrent intake of other micronutrients relevant to fetal growth. However, most micronutrients return to normal fairly rapid after delivery;[22,41] in addition, from the available follow-up information, which included a retrospective assessment of the sort of supplements used (single supplements vs. multivitamin supplements; available for 1509 of the 2029 supplement users in this study) it can be concluded that the majority were single users only (65%). In this context, we assume the role of nutrients other than folate to be minor.

A strength of our study is the focus on term birth weight and SGA in parallel. SGA is the most important outcome in terms of clinical relevance, but the use of birth weight as continuous measure and the planned exclusion of preterm births allowed us to also capture the physiological evidence on overall weight effects of folate, without the distorting effect of preterm delivery.[42] The mechanism through which folate depletion affects preterm delivery is likely to differ from the mechanism through which it affects birth weight, further justifying this separation. The observed corresponding effects of interpregnancy interval on term birth weight and SGA in this cohort were adjusted for a large set of simultaneously measured risk factors relevant to fetal growth in the index pregnancy. However, the design of our study limited our ability to measure factors related to the preceding pregnancy, such as the outcome of pregnancy and maternal breastfeeding. Breastfeeding practices may interact with the association between folate depletion and interpregnancy interval, as women who breast-feed are more at risk of depletion.[20] The outcome of the preceding pregnancy has been shown to relate to the outcome of subsequent pregnancies.[43] However, this correlation may reflect one or more persisting factors that affect all infants in a sibship, such as smoking.[44] To the extent that these factors were known in our cohort, they were adjusted for in our analyses. In addition, in previous studies that did control for the outcome of the preceding pregnancy, the adjustment did not affect the J-shaped relationship between interpregnancy interval and risk of fetal growth restriction.[10-12] Another limitation related to the design of our study may be the measurement of folic acid supplement use, which was self-reported. One could assume that over-reporting of this social desirable healthy behavior influenced our observations. However, self-administered questionnaires have been shown to validly measure folic acid use;[45] if any over-reporting would be present, this might imply that our estimates are too conservative.

Throughout the world, the use of folic acid supplements as proven measure to prevent birth defects has induced specific public health policies to promote folic acid intake.[46,47] While the issue presented here is relevant for the majority of countries that have supplementation policies similar to those in the Netherlands, it is more difficult to generalize our results to countries where supplementation policies are integrated with the population-wide fortification of flour, such as in the Unites States.[46] However, recent evidence from the US has shown the

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persistent need for supplementation, as after an initial rise folate concentrations in women of childbearing age have declined again in the recent years.[48] while low levels are still common among ethnic minority groups, who also are at risk for short interpregnancy intervals.[4-7,9-12]

In summary, this large-scale prospective cohort study suggests that folate depletion contributes to the consistent association between short interpregnancy intervals and fetal growth restriction. One could argue that the evidence is sufficient to carry through universal postpartum supplementation – also for the maternal benefit of quick and adequate restoration of folate stores. However, given the current debate on the longer term benefits and risks of folic acid supplementation in pregnancy,[47] caution is warranted. Only prospective postnatal intervention studies that also include other relevant outcome measures (such as preterm delivery) can provide decisive evidence in this regard. Meanwhile, one should keep in mind that primary prevention of the interpregnancy interval-associated risks still rests on family planning education, for which the best window of opportunity may lie in the early postnatal period – a critical stage for identifying those women who, intentionally or unintentionally, are most likely to have a short interpregnancy interval.

Acknowledgements

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References

1. Fedrick J, Adelstein P. Influence of pregnancy spacing on outcome of pregnancy. Br Med J 1973;4:753–6.
2. Ferraz EM, Gray RH, Fleming PL, Maia TM. Interpregnancy interval and low birth weight: findings from a case-control study. Am J Epimiol 1988;128:1111–6.
3. Lieberman E, Lang JM, Ryan KJ, Monson RR, Schoenbaum SC. The association of inter-pregnancy interval with small for gestational age births. Obstet Gyn 1989;74:1–5.
4. Rawlings JS, Rawlings VB, Read JA. Prevalence of low birth weight and preterm delivery in relation to the interval between pregnancies among white and black women. N Engl J Med 1995;332:69–74.
5. Kallan JE. Reexamination of interpregnancy intervals and subsequent birth outcomes: evidence from U.S. linked birth/infant death records. Soc Biol 1997;44:205–12.
6. Adams MM, Delaney KM, Stupp PW, McCarthy BJ, Rawlings JS. The relationship of interpregnancy interval to infant birthweight and length of gestation among low-risk women, Georgia. Paediatr Perinat Epidemiol 1997;11(suppl 1):48–62.
7. Khoshnood B, Lee KS, Wall S, Hsieh HL, Mittendorf R. Short interpregnancy intervals and the risk of adverse birth outcomes among five racial/ethnic groups in the United States. Am J Epidemiol 1998;148:798–805.
8. Shults RA, Arndt V, Olshan AF, Martin CF, Royce RA. Effects of short interpregnancy intervals on small-for-gestational age and preterm births. Epidemiology 1999;10:250–4.
9. James AT, Bracken MB, Cohen AP, Saftlas A, Lieberman E. Interpregnancy interval and disparity in term small for gestational age births between black and white women. Obstet Gynecol 1999;93:109–12.
10. Zhu BP, Rolfs RT, Nangle BE, Horan JM. Effect of the interval between pregnancies on perinatal outcomes. N Engl J Med 1999;340:589–94.
11. Zhu BP, Haines KM, Le T, McGrath-Miller K, Boulton ML. Effect of the interval between pregnancies on perinatal outcomes among white and black women. Am J Obstet Gynecol 2001;185:1403–10.
12. Zhu BP, Le T. Effect of interpregnancy interval on infant low birth weight: a retrospective cohort study using the Michigan Maternally Linked Birth Database. Matern Child Health J 2003;7:169–78.
13. Conde-Agudelo A, Belizan JM, Norton MH, Rosas-Bermudez A. Effect of the interpregnancy interval on perinatal outcomes in Latin America. Obstet Gynecol 2005;106:359–66.
14. Conde-Agudelo A, Rosas-Bermudez A, Kafury-Goeta AC. Birth spacing and risk of adverse perinatal outcomes: a meta-analysis. JAMA 2006;295:1809–23.
15. Erickson JD, Bjerkedal T. Interpregnancy interval. Association with birth weight, stillbirth, and neonatal death. J Epidemiol Community Health 1978;32:124–30.
16. Klebanoff MA. Short interpregnancy interval and the risk of low birth weight. Am J Public Health 1988;78:667–70.
17. Klebanoff MA. The interval between pregnancies and the outcome of subsequent births. N Engl J Med 1999;340:643–4.
18. Winkvist A, Rasmussen KM, Habicht JP. A new definition of maternal depletion syndrome. Am J Public Health 1992;82:691–4.
19. King JC. The risk of maternal nutritional depletion and poor outcomes increases in early or closely spaced pregnancies. J Nutr 2003;133:1732S–6S.

20. Smits LJ, Essed GG. Short interpregnancy intervals and unfavourable pregnancy outcome: role of folate depletion. Lancet 2001;358:2074–7.
21. Milman N, Byg KE, Hvas AM, Bergholt T, Eriksen L. Erythrocyte folate, plasma folate and plasma homocysteine during normal pregnancy and postpartum: a longitudinal study comprising 404 Danish women. Eur J Haematol 2006;76:200–5.
22. Bruinse HW, van den Berg H. Changes of some vitamin levels during and after normal pregnancy. Eur J Obstet Gynecol Reprod Biol 1995;61:31–7.
23. Green R, Miller JW. Folate deficiency beyond megaloblastic anemia: hyperhomocysteinemia and other manifestations of dysfunctional folate status. Semin Hematol 1999;36:47–64.
24. Tamura T, Goldenberg RL, Freeberg LE, Cliver SP, Cutter GR, Hoffman HJ. Maternal serum folate and zinc concentrations and their relationships to pregnancy outcome. Am J Clin Nutr 1992;56:365–70.
25. Scholl TO. Hediger ML, Schall JI, Khoo CS, Fischer RL. Dietary and serum folate: their influence on the outcome of pregnancy. Am J Clin Nutr 1996;63:520–5.
26. Sram RJ, Binkova B, Lnenickova Z, Solansky I, Dejmek J. The impact of plasma folate levels of mothers and newborns on intrauterine growth retardation and birth weight. Mutat Res 2005;591:302–10.
27. Vollset SE, Refsum H, Irgens LM, et al. Plasma total homocysteine, pregnancy complications, and adverse pregnancy outcomes: the Hordaland Homocysteine Study. Am J Clin Nutr 2000;71:962–8.
28. Murphy MM, Scott JM, Arija V, Molloy AM, Fernandez-Ballart JD. Maternal homocysteine before conception and throughout pregnancy predicts fetal homocysteine and birth weight. Clin Chem 2004;50:1406–12.
29. Lindblad B, Zaman S, Malik A, et al. Folate, vitamin B12, and homocysteine levels in South Asian women with growth-retarded fetuses. Acta Obstet Gynecol Scand 2005;84:1055–61.
30. van Eijsden M, van der Wal MF, Bonsel GJ. Folic acid knowledge and use in a multi-ethnic pregnancy cohort: the role of language proficiency. BJOG 2006;113:1446–51.
31. van der Pal-de Bruin KM, de Walle HE, Jeeninga W, et al. The Dutch 'Folic Acid Campaign'--have the goals been achieved? Paediatr Perinat Epidemiol 2000;14:111–7.
32. Allison PD. Missing data. Thousand Oaks, CA: Sage Publications, 2001.
33. Radloff LS. The CES-D scale: a self-report depression scale for research in the general population. Applied Psychological Measurement 1977;1:385–401.
34. Spielberger CD, Gorsuch RL, Lushene RE. STAI manual for the State-Trait Anxiety Inventory ("self-evaluation questionnaire"). Palo Alto, CA: Consulting Psychologists Press, 1970.
35. Huizink AC. Mulder EJ, Robles de Medina PG, Visser GH, Buitelaar JK. Is pregnancy anxiety a distinctive syndrome? Early Hum Dev 2004;79:81–91.
36. Crnic KA, Greenberg MT. Minor parenting stresses with young children. Child Dev 1990;61:1628–37.
37. Karasek R, Brisson C, Kawakami N, Houtman I, Bongers P, Amick B. The Job Content Questionnaire (JCQ): an instrument for internationally comparative assessments of psychosocial job characteristics. J Occup Health Psychol 1998;3:322–55.
38. Scholl TO, Johnson WG. Folic acid: influence on the outcome of pregnancy. Am J Clin Nutr 2000;71(suppl 5):1295S–303S.

39. Rao S, Yajnik CS, Kanade A, et al. Intake of micronutrient-rich foods in rural Indian mothers is associated with the size of their babies at birth: Pune Maternal Nutrition Study. J Nutr 2001;131:1217–24.
40. Baumslag N, Edelstein T, Metz J. Reduction of incidence of prematurity by folic acid supplementation in pregnancy. Br Med J 1970;1:16–17.
41. Cikot RJ, Steegers-Theunissen RP, Thomas CM, de Boo TM, Merkus HM, Steegers EA. Longitudinal vitamin and homocysteine levels in normal pregnancy. Br J Nutr 2001;85:49–58.
42. Wilcox AJ. On the importance – and the unimportance – of birthweight. Int J Epidemiol 2001;30:1233–41.
43. Melve KK, Skjaerven R, Øyen N. Families with a perinatal death: is there an association between the loss and the birthweight of surviving siblings? Paediatr Perinat Epidemiol 2002;16:23–32.
44. Nordström ML, Cnattingius S. Smoking habits and birthweights in two successive births in Sweden. Early Hum Dev 1994;37:195–204.
45. Burton A, Wilson S, Gillies AJ. Folic acid: is self reported use of supplements accurate? J Epidemiol Community Health 2001;55:841–2.
46. Botto LD, Lisi A, Robert-Gnansia E, et al. International restrospective cohort study of neural tube defects in relation to folic acid recommendations: are the recommendations working. BMJ 2005;330:571.
47. Cornel MC, de Smit DJ, de Jong-van den Berg LT. Folic acid – the scientific debate as a base for public health policy. Reprod Toxicol 2005;20:411–5.
48. Centers for Disease Control and Prevention (CDC). Folate status in women of childbearing age, by race/ethnicity – United States 1999-2000, 2001-2002, and 2003-2004. MMWR Morb Mort Wkly Rep 2007;55:1377–80.

Chapter 7

MATERNAL N-3, N-6, AND *TRANS* FATTY ACID PROFILE EARLY IN PREGNANCY AND TERM BIRTH WEIGHT: A PROSPECTIVE COHORT STUDY

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Abstract

Background

Maternal n-3, n-6, and *trans* fatty acids are claimed to affect fetal growth, yet evidence is limited.

Objective

We investigated the association between maternal n-3, n-6, and *trans* fatty acids measured early in pregnancy and fetal growth.

Design

Amsterdam pregnant women (n = 12 373) were invited to complete a questionnaire (response 67%) and donate blood around the 12th pregnancy week for nutrient analysis. For 4336 women, fatty acid concentrations were measured in plasma phospholipids (gas-liquid chromatography). Associations of these concentrations with birth weight and small-for-gestational-age (SGA) risk were analyzed (liveborn singleton term deliveries, n = 3704).

Results

Low concentrations of individual n-3 fatty acids and 20:3n-6, the precursor of arachidonic acid (20:4n-6), but high concentrations of the other n-6 fatty acids and the main dietary *trans* fatty acid (18:1n-9*t*) were associated with lower birth weight (estimated difference in univariate analysis −52 to −172 g for extreme quintile compared with middle quintile). In general, SGA risk increased accordingly. After adjustment for physiologic, lifestyle-related, and sociodemographic factors, low concentrations of most n-3 fatty acids and 20:3n-6 and high concentrations of 20:4n-6 remained associated with lower birth weight (−52 to −57 g), higher SGA risk (odds ratios: 1.38 to 1.50), or both. Infants of the 7% of women with the most adverse fatty acid profile were on average 125 g lighter and twice as likely to be small for gestational age.

Conclusion

An adverse maternal fatty acid profile early in pregnancy is associated with reduced fetal growth, which, if confirmed, gives perspective for the dietary prevention of lower birth weight.

Introduction

The pregnancy outcomes low birth weight (<2500 g) and small for gestational age (SGA; <10th percentile of reference population) that are due to intrauterine growth restriction are major determinants of children's future health and development. At the short term, infants born with low birth weight or SGA are at increased risk of infant mortality and morbidity,[1] while at the long term, they have higher risks of metabolic and cardiovascular diseases.[2,3]

Although adequate maternal nutrition has since long been accepted as vital to fetal growth, it is still largely unclear to what extent which (combinations of) nutrients matter.[4,5] Consequently, no evidence-based nutritional preventive strategies exist at this stage. Among the nutrients with potential clinical relevance, the essential fatty acids α-linolenic acid (18:3n-3) and linoleic acid (18:2n-6), and more particular their longer-chain, more-unsaturated derivatives (commonly referred to as long-chain polyunsaturated fatty acids, LC-PUFAs) have increasingly gained interest. LC-PUFAs are key components of virtually all cellular membranes and, besides, exert a wide array of biological functions.[6,7] Although LC-PUFAs are considered essential for fetal growth, evidence of their growth-promoting effect is limited. Results of the few small-sized observational studies that directly measured n-3 and n-6 fatty acid concentrations, either maternal,[8] neonatal,[9] or both,[10-12] are inconclusive. Evidence from existing randomized clinical trials is restricted to maternal n-3 LC-PUFA intake only; although positive associations are reported, these are commonly interpreted as the consequence of a prolonged gestation rather than a direct effect on fetal growth.[13,14]

In the present large cohort study, we explore the potential role of the maternal n-3 and n-6 fatty acid profile in fetal growth by investigating in detail the association between maternal concentrations of these fatty acids during early pregnancy and infant birth weight at term. We also explore the role of elaidic acid (18:1n-9*t*), the main industrial *trans* fatty acid in the diet, which can inhibit the conversion of α-linolenic acid and linoleic acid to their respective LC-PUFAs and was suggested to inhibit fetal growth.[15] The simultaneous measurement of a predefined set of cofactors relevant to fetal growth enables us to investigate the independent association of these maternal diet-derived fatty acids with infant birth weight. If the early pregnancy fatty acid profile proves to be relevant, this may open new avenues for optimizing infant development and disease prevention by adaptation of the maternal fatty acid status.

Subjects and methods

Study population and design

This study is part of the Amsterdam Born Children and their Development (ABCD) study, a prospective community-based cohort study that examines the relationship between maternal lifestyle and psychosocial conditions during pregnancy and the child's health at birth as well

as in later life. Essentials of the study design were described previously.[16] In short, between January 2003 and March 2004, all pregnant women living in Amsterdam were invited to enroll in the ABCD study at their first prenatal visit to the obstetric care provider (around the 12th week of gestation) and were requested to complete a questionnaire, covering socio-demographic data, obstetric history, lifestyle, dietary habits, and psychosocial factors. The questionnaire was available in Dutch and in English, Turkish, or Arabic for immigrant women. In addition, women were invited to participate in the ABCD biomarker study. For this, an additional blood sample was taken during routine blood collection for screening purposes after the first prenatal checkup.

Of the invited 12 373 pregnant women, 8266 returned the pregnancy questionnaire (response rate 67%). Of those respondents, 53% (n = 4389) participated in the biomarker study. Approval of the study was obtained from the Medical Ethical Committees of participating hospitals and the Registration Committee of Amsterdam. Written informed consent was obtained from all participants.

Measurements

Primary outcome variables for this study were birth weight (in grams) and SGA (yes, no) at term, with SGA defined as a birth weight below the 10th percentile for gestational age on the basis of sex- and parity-specific standards from the Netherlands Perinatal Registry (data available from the authors upon request). Date of birth, birth weight, infant sex, and gestational age [ultrasound-based or, if unavailable (<10%), based on time of last menstrual period], as recorded by the obstetric care providers, were obtained through the Youth Health Department at the Municipal Health Service in Amsterdam. Gestational age at blood sampling was calculated with the available information on gestational age at birth, date of birth, and date of blood sampling. Information on maternal physiologic, lifestyle and sociodemographic characteristics was obtained from the questionnaire.

The sociodemographic covariables included cohabitant status (living together with partner, not living together/single), educational attainment after primary school (≤5, 6–10, ≥11 y), and ethnicity. Ethnicity was defined by country of birth and included the following categories, based on the Dutch main ethnic populations: the Netherlands, Surinam, Turkey, Morocco, other non-Western country, and other Western country.

Lifestyle factors included self-reported alcohol consumption (last week's consumption, recoded into yes, no), self-reported smoking (last week's behavior, recoded into yes, no), pre-gravid body mass index (BMI, in kg/m^2) based on self-reported height and weight, and psychosocial stress (presence of 0, 1, or ≥2 stressors). A random imputation procedure with the use of linear regression analysis was used to complete missing data on height (3.8% missing) or weight (9.9% missing).[17] Psychosocial stressors were measured by validated Dutch versions of internationally accepted questionnaires and included depression,[18] general anxiety,[19] pregnancy-related anxiety,[20] parenting stress,[21] and work stress.[22] For all scales, thresholds

for nonnormal scores were, in absence of internationally agreed cutoff points for pregnant women, chosen at the 90th percentile. Self-reported maternal physiologic characteristics were parity (0, ≥1), age (≤24, 25–34, and ≥35 y) and height.

Biochemical analyses

For each participant of the biomarker study, one blood sample was taken in a 10-mL EDTA(K2) evacuated tube (Vacutainer; Becton Dickinson BV, Alphen aan de Rijn, The Netherlands) and sent to the Regional Laboratory of Amsterdam for processing. Transport was by courier or by overnight mail in special envelopes, enabling processing within 28 h of sampling. A previous study of our group showed that this delay did not compromise the validity of measured biomarkers.[23] At the laboratory, plasma was prepared by centrifugation (1600 x *g* for 10 min at room temperature) and stored as 1-mL aliquots at −80°C until analysis.

Fatty acid analysis was performed at the Analytical Biochemical Laboratory (ABL, Assen, the Netherlands), as previously described.[24,25] In short, after the addition of an internal standard (1,2-dinonadecanoyl-*sn*-glycero-3-phosphocholine) and 10-heptadecenoic acid (17:1) to check for carryover of free fatty acids during the isolation procedure, plasma lipid extracts were prepared by a modified Folch extraction method[26] after which phospholipids were isolated by solid-phase extraction on aminopropyl-silica columns (500 mg/3 mL, Varian, Palo Alto, CA, USA).[27] Phospholipids were then hydrolyzed and the resulting fatty acids were methylated with boron trifluoride-methanol.[28] Finally, the fatty acid methyl esters were separated and quantified by capillary gas chromatography with flame ionization detection (HP5890 series II, Hewlett Packard, Palo Alto, CA, USA), with the use of a polar and a nonpolar column (BPx70 and BP1, respectively, SGE Analytical Science Pty. Ltd, Ringwood, Victoria, Australia). The oven temperature was programmed to begin at 160°C for 4 minutes, and then to increase to 200°C by 6.0 °C/min. After 3 minutes, the temperature was further increased to 260°C at a rate of 7 °C/min and kept constant for 2.34 min. The injector temperature was kept at 250°C and the detector temperature at 300°C.

Absolute amounts of fatty acids (in mg/L plasma) were quantified on the basis of recovery from the internal standard and calculated in relative values (percentage of total fatty acids). For the present study, the following fatty acids were relevant and will be presented: the *trans* fatty acid elaidic acid (18:1n-9*t*), the essential fatty acids α-linolenic acid (18:3n-3) and linoleic acid (18:2n-6), and their respective LC-PUFAs eicosatetraenoic acid (20:4n-3), eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3), docosahexaenoic acid (DHA, 22:6n-3), dihomo-γ-linolenic acid (DGLA, 20:3n-6), arachidonic acid (AA, 20:4n-6), adrenic acid (22:4n-6) and Osbond acid (22:5n-6). Interassay coefficients of variation for these fatty acids varied from ≤22% (for 20:4n-3, the fatty acid with the lowest concentration) to ≤2% (for 18:2n-6, the fatty acid with the highest concentration). The essential fatty acid derivatives γ-linolenic acid (18:3n-6) and stearidonic acid (18:4n-3) were not included, because their concentrations were <0.1% of total fatty acids. Other measured, nonessential fatty acids were not considered because they were outside the scope of our study.

Statistical analysis

Fatty acid results were available for 4336 of the 4389 participants. From this group, 4112 women gave birth to a liveborn singleton infant for whom information on birth weight and gestational age was available. We excluded all respondents with known diabetes (n = 21) or hypertension (n = 127) at the time of blood sampling, respondents who delivered preterm (n = 213), and respondents with missing values on ≥1 of above-mentioned covariables (n = 55). The final sample available for analysis was 3704.

After a descriptive analysis of fatty acid concentrations, outcome variables, and maternal and infant characteristics, the univariate (i.e., unadjusted) associations between individual maternal fatty acid concentrations and infant birth weight were explored by linear regression analyses. For each fatty acid, 2 separate models were explored: (1) a continuous model, which included the SD-score as continuous measure of fatty acid concentrations, and (2) a categorical model, which included quintiles as categorical measure of fatty acid concentrations. The categorical model was chosen to explore the potential nonlinearity of the association; with the use of the middle quintile as reference, this analysis allowed us to examine whether associations were apparent over the full exposure distribution or at the extremes only, as observed before for the n-3 LC-PUFAs.[29] Subsequently, multivariate analyses with the use of a stepwise hierarchical approach were performed to assess the adjusted contributions of the individual fatty acids. In the first step (model 1) we included maternal physiologic characteristics (see *Measurements*), infant sex, gestational age at birth, and gestational age at blood sampling. The latter covariate was included to standardize for changes in fatty acid concentration that normally occur during pregnancy.[24] In the second step (model 2) we subsequently added all lifestyle and sociodemographic factors. Finally, to assess the association between the overall maternal fatty acid profile and birth weight, thereby taking into account their metabolic interrelations,[6,30] we calculated a cumulative exposure score. For each fatty acid a dichotomous (0,1) classification of exposure was determined on the basis of its univariate association with birth weight. For each fatty acid positively associated with birth weight (i.e., for 6 fatty acids, see *Results*) the lowest quintile was scored as 1 (exposure); for each fatty acid negatively associated with birth weight (5 fatty acids), the highest quintile was scored as 1. After summation of the scores for each of the 11 fatty acids (the so-called cumulative exposure score), multivariate linear regression analysis was performed to explore the association between this cumulative exposure score and birth weight. To allow for sufficiently sized groups for comparison, scores were combined into 4 categories: 0–1, 2–3, 4–5, and ≥6, with the latter category defined as the most adverse profile.

All regression analyses were repeated using logistic regression to explore the associations between maternal fatty acid status and SGA. Associations were considered significant at $P < 0.05$. All analyses were conducted in SPSS version 14.0 (SPSS Inc., Chicago, IL, USA).

Results

Characteristics of the included mother-infant pairs (n = 3704) are presented in **Table 7.1**; maternal fatty acid concentrations with quintile distributions are shown in **Table 7.2**. Blood samples were taken at a mean (± SD) gestational duration of 13.5 ± 3.3 wk. Compared with the infants born to mothers who did not provide a blood sample (n = 3564; data not shown), the infants included in the present study were more often of the female sex, less often born SGA and had higher birth weights. The included mothers were older, taller, and higher educated; had a lower BMI; experienced less stress; and were more likely to consume alcohol. They were also more often nulliparous and of Dutch origin and were less often living alone.

Univariate regression results showed that birth weight was positively related to all n-3 fatty acids as well as the n-6 LC-PUFA DGLA (20:3n-6), but negatively related to all other n-6 fatty acids and elaidic acid (18:1n-9*t*) (**Table 7.3**). The categorical model showed these associations to be present at the extremes of the exposure scale only. Compared with infants born to women with intermediate concentrations, infants born to women in the lowest quintile of α-linolenic acid (18:3n-3), eicosatetraenoic acid (20:4n-3), EPA (20:5n-3), DPA (22:5n-3), DHA (22:6n-3), or DGLA (20:3n-6) had birth weights 56–172 g lower; infants of women in the highest quintile of linoleic acid (18:2n-6), AA (20:4n-6), adrenic acid (22:4n-6), Osbond acid (22:5n-6), or elaidic acid (18:1n-9*t*) had birth weights 52–90 g lower.

Given the nonlinearity of the univariate results, multivariate analyses were restricted to the categorical model. Overall, adjustment did not change directions of the associations. Adjustment for physiologic factors (model 1) however largely attenuated the estimates, whereas additional adjustment for lifestyle and sociodemographic factors (model 2) showed less effect. After full adjustment, the n-3 LC-PUFA EPA and the n-6 LC-PUFAs DGLA and AA were still significantly associated with birth weight (estimated difference compared with reference: −55 and −57 grams for lowest quintiles of EPA and DGLA, respectively, and −52 grams for the highest quintile of AA).

Results for SGA were largely similar. The univariate categorical models demonstrated increased risks of SGA at lower concentrations of most n-3 fatty acids (exception: α-linolenic acid and DHA) and the n-6 fatty acid DGLA, and at higher concentrations of most other n-6 fatty acids (exception: Osbond acid) (**Table 7.4**). Again, adjustment did not change directions of the associations but attenuated the univariate estimates. After full adjustment (model 2), significantly increased risks of delivering an infant that was SGA were still observed for women with the lowest concentrations of n-3 eicosatetraenoic acid [odds ratio (OR): 1.50; 95% CI: 1.07, 2.11] and DPA (OR: 1.49; 95% CI: 1.06, 2.10). For DGLA, the largest increase in risk was observed for women in the 2 lowest quintiles; however, the increase was only significant for women in the second quintile (OR: 1.38; 95% CI: 1.00, 1.91).

Table 7.1 Infant and maternal characteristics in the study population (n = 3704)

Characteristic	Value
Infant sex (% male)	48.8
Small-for-gestational-age birth (%)	11.6
Birth weight (g)	3509 ± 485[a]
Gestational age at birth (wk)	39.7 ± 1.2
Gestational age at blood sampling (wk)	13.5 ± 3.3
Maternal age (%)	
≤24 y	11.2
25–34 y	66.5
≥35 y	22.3
Parity (% nullipara)	57.6
Maternal height (cm)	169.1 ± 7.1
Maternal pregravid BMI (kg/m^2)	22.7 ± 3.5
Alcohol consumer (%)	25.3
Smoking (%)	9.2
Psychosocial stressors (%)	
0	76.1
1	15.0
≥2	9.0
Cohabitant status (% single or living alone)	11.2
Education (%)	
≤5 y	17.0
6–10 y	38.4
≥11 y	44.6
Country of birth (%)	
The Netherlands	70.0
Surinam	4.0
Turkey	3.4
Morocco	5.2
Other non-Western country	8.9
Other Western country	8.5

[a] *Mean ± SD (all such values).*

The multivariate exposure score analysis showed that the largest decreases in birth weight and increases in risk of SGA were observed for the ≈20% women with scores ≥4. Infants born to these mothers were on average 72 g lighter and 1.5 times (95% CI: 1.14, 2.06 times) more likely to be SGA than infants born to mothers with the lowest score. Infants born to the 7% mothers with the most adverse fatty acid profile (score ≥6) were 125 g lighter and twice as likely to be SGA (95% CI: 1.44, 3.13) (**Table 7.5**).

Table 7.2 Concentrations of selected fatty acids by quintile (Q) in maternal plasma phospholipids at early pregnancy (n = 3704)[a]

Fatty acid	Mean value	Quintile distribution				
		Q1	Q2	Q3	Q4	Q5
Total fatty acids (mg/L)	1443.89 ± 239.43[b]	≤1242.64	1243.64–1372.82	1372.82–1490.78	1490.78–1628.62	>1628.62
Fatty acid fractions[c]						
α-Linolenic acid (18:3n-3)	0.18 ± 0.08	≤0.11	0.11–0.15	0.15–0.18	0.18–0.23	>0.23
Eicosatetraenoic acid (20:4n-3)	0.14 ± 0.06	≤0.08	0.08–0.11	0.11–0.14	0.14–0.18	>0.18
EPA (20:5n-3)	0.63 ± 0.44	≤0.34	0.34–0.46	0.46–0.58	0.58–0.80	>0.80
DPA (22:5n-3)	0.73 ± 0.19	≤0.57	0.57–0.67	0.67–0.76	0.76–0.88	>0.88
DHA (22:6n-3)	4.79 ± 1.10	≤3.85	3.85–4.47	4.47–5.00	5.00–5.67	>5.67
Linoleic acid (18:2n-6)	19.07 ± 2.50	≤16.95	16.95–18.37	18.37–19.65	19.65–21.08	>21.08
DGLA (20:3n-6)	3.41 ± 0.74	≤2.78	2.78–3.18	3.18–3.53	3.53–4.01	>4.01
AA (20:4n-6)	9.25 ± 1.62	≤7.90	7.90–8.77	8.77–9.58	9.58–10.58	>10.58
Adrenic acid (22:4n-6)	0.38 ± 0.11	≤0.29	0.29–0.34	0.34–0.39	0.39–0.46	>0.46
Osbond acid (22:5n-6)	0.37 ± 0.16	≤0.24	0.24–0.31	0.31–0.38	0.38–0.48	>0.48
Elaidic acid (18:1n-9t)	0.23 ± 0.10	≤0.15	0.15–0.18	0.18–0.22	0.22–0.29	>0.29

[a] *EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid.*

[b] *Mean ± SD (all such values).*

[c] *Relative concentrations (% of total fatty acids).*

Table 7.3 Association between maternal fatty acid concentrations in plasma phospholipids [SD scores and quintiles (Q)] and birth weight[a]

			Univariate model[b]		Multivariate model[b]	
Fatty acid	**n**	**Birth weight[c]**	Continuous[d]	Categorical[e]	Model 1[e,f]	Model 2[e,g]
α-Linolenic acid (18:3n-3)			21.7 ± 8.0*			
Q1	595	3465 ± 470		−55.7 ± 27.4[§]	−5.4 ± 23.8	−14.0 ± 23.8
Q2	812	3507 ± 484		−13.5 ± 25.4	12.9 ± 21.8	8.1 ± 21.5
Q3	661	3521 ± 501		0.0	0.0	0.0
Q4	873	3509 ± 483		−11.6 ± 25.0	−11.3 ± 21.4	−8.4 ± 21.0
Q5	763	3537 ± 485		16.0 ± 25.8	8.6 ± 22.1	12.1 ± 21.7
Eicosatetraenoic acid (20:4n-3)			40.7 ± 7.9*			
Q1	623	3409 ± 491		−129.6 ± 25.9*	−54.7 ± 23.2*	−46.6 ± 24.0
Q2	728	3487 ± 469		−52.0 ± 24.8[§]	−15.4 ± 21.5	−13.9 ± 21.2
Q3	787	3539 ± 496		0.0	0.0	0.0
Q4	793	3530 ± 492		−8.4 ± 24.3	−14.4 ± 20.9	−13.1 ± 20.6
Q5	773	3560 ± 465		21.5 ± 24.4	22.9 ± 21.0	28.1 ± 20.7
EPA (20:5n-3)			22.1 ± 8.0*			
Q1	694	3389 ± 479		−171.6 ± 25.7*	−63.2 ± 24.0*	−54.6 ± 24.2[§]
Q2	762	3511 ± 497		−48.4 ± 25.1	−13.5 ± 21.9	−5.8 ± 21.6
Q3	715	3560 ± 471		0.0	0.0	0.0
Q4	784	3542 ± 492		−18.0 ± 24.9	−17.5 ± 21.5	−19.1 ± 21.1
Q5	749	3536 ± 467		−24.4 ± 25.2	−14.3 ± 21.8	−19.4 ± 21.5
DPA (22:5n-3)			33.7 ± 8.0*			
Q1	724	3435 ± 486		−89.6 ± 25.5*	−18.8 ± 22.9	−27.2 ± 23.1
Q2	749	3501 ± 494		−23.0 ± 25.3	1.1 ± 21.8	−3.6 ± 21.5
Q3	717	3525 ± 484		0.0	0.0	0.0
Q4	769	3537 ± 483		12.1 ± 25.1	−9.8 ± 21.7	−9.6 ± 21.3
Q5	745	3547 ± 472		22.7 ± 25.3	−5.0 ± 21.9	0.6 ± 21.5
DHA (22:6n-3)			23.6 ± 8.0*			
Q1	732	3459 ± 494		−94.5 ± 25.2*	−62.8 ± 21.8*	−43.0 ± 21.9
Q2	746	3482 ± 504		−71.4 ± 25.1*	−46.5 ± 21.6[§]	−31.8 ± 21.3
Q3	740	3554 ± 478		0.0	0.0	0.0
Q4	739	3512 ± 474		−42.0 ± 25.2	−29.6 ± 21.6	−21.7 ± 21.3
Q5	747	3539 ± 469		−14.5 ± 25.1	−6.6 ± 21.6	−5.2 ± 21.3
Linoleic acid (18:2n-6)			−29.0 ± 8.0*			
Q1	738	3537 ± 480		24.4 ± 25.2	7.8 ± 21.6	−0.1 ± 21.4
Q2	740	3535 ± 499		21.8 ± 25.2	13.7 ± 21.6	11.0 ± 21.2
Q3	741	3513 ± 483		0.0	0.0	0.0
Q4	743	3517 ± 489		4.6 ± 25.1	11.9 ± 21.6	17.2 ± 21.2
Q5	742	3446 ± 469		−67.2 ± 25.1*	−46.4 ± 21.7[§]	−30.5 ± 21.5

(continued)

Table 7.3 (continued)

			Univariate model[b]		Multivariate model[b]	
Fatty acid	**n**	**Birth weight[c]**	Continuous[d]	Categorical[e]	Model 1[e,f]	Model 2[e,g]
DGLA (20:3n-6)			45.9 ± 7.9*			
Q1	737	3416 ± 448		−116.4 ± 25.1*	−68.8 ± 21.6*	−56.7 ± 21.5*
Q2	737	3499 ± 499		−33.8 ± 25.1	−29.8 ± 21.6	-21.1 ± 21.2
Q3	742	3532 ± 491		0.0	0.0	0.0
Q4	740	3555 ± 489		22.6 ± 25.1	21.0 ± 21.5	13.7 ± 21.2
Q5	748	3545 ± 484		12.4 ± 25.0	16.9 ± 21.5	14.5 ± 21.2
AA (20:4n-6)			−22.6 ± 8.0*			
Q1	740	3513 ± 467		−37.2 ± 25.2	−7.2 ± 21.7	10.8 ± 21.4
Q2	736	3519 ± 483		−31.1 ± 25.2	−27.9 ± 21.7	−23.8 ± 21.3
Q3	737	3550 ± 492		0.0	0.0	0.0
Q4	747	3505 ± 491		−45.3 ± 25.2	−31.6 ± 21.6	−40.2 ± 21.2
Q5	744	3460 ± 489		−90.0 ± 25.2*	−44.9 ± 21.8[§]	−51.6 ± 21.8[§]
Adrenic acid (22:4n-6)			−34.0 ± 8.0*			
Q1	687	3551 ± 476		33.2 ± 25.7	34.5 ± 22.1	32.0 ± 21.8
Q2	690	3506 ± 481		−11.7 ± 25.7	−7.6 ± 22.1	−13.2 ± 21.7
Q3	728	3518 ± 474		0.0	0.0	0.0
Q4	799	3529 ± 486		11.0 ± 24.8	34.1 ± 21.3	35.9 ± 21.0
Q5	800	3449 ± 500		−69.5 ± 24.8*	−20.7 ± 21.5	−8.9 ± 21.4
Osbond acid (22:5n-6)			−27.7 ± 8.0*			
Q1	722	3512 ± 465		−16.7 ± 25.8	−25.8 ± 22.2	−27.2 ± 21.8
Q2	750	3521 ± 483		−7.9 ± 25.6	−20.3 ± 21.9	−16.8 ± 21.6
Q3	690	3529 ± 496		0.0	0.0	0.0
Q4	758	3533 ± 483		4.4 ± 25.5	15.3 ± 21.9	24.2 ± 21.5
Q5	784	3456 ± 495		−73.3 ± 25.3*	−32.2 ± 22.0	−16.0 ± 21.8
Elaidic acid (18:1n-9*t*)			−26.1 ± 8.0*			
Q1	637	3519 ± 453		−0.7 ± 25.6	11.0 ± 22.1	5.6 ± 22.0
Q2	685	3538 ± 480		17.8 ± 25.1	12.5 ± 21.6	9.7 ± 21.2
Q3	814	3520 ± 505		0.0	0.0	0.0
Q4	824	3505 ± 484		−15.1 ± 24.0	−15.3 ± 20.6	−7.2 ± 20.2
Q5	744	3468 ± 493		−51.9 ± 24.6[§]	−26.7 ± 21.2	−14.2 ± 20.9

*[a] Linear regression analysis with birth weight as dependent variable and fatty acid concentration as independent variable. Q1 through Q5 represents the quintile distribution of the relative concentrations as given in Table 7.2. EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid; [b] All values are B ± SD; [c] All values in this column are mean ± SD; [d] Continuous model with the SD score as measure of the fatty acid concentration, B ± SE is the unstandardized regression coefficient, representing the change in birth weight associated with a 1-SD increase in fatty acid concentration; [e] Categorical model with quintiles as measure of the fatty acid concentration; B ± SE is the unstandardized regression coefficient, representing the difference between the birth weight in the specific quintile and that in the reference quintile (Q3); [f] Adjusted for gestational age at blood sampling, gestational age at birth (linear and quadratic), infant sex, maternal age, height, and parity; [g] As in model 1 with additional adjustment for maternal pregravid BMI (linear and quadratic), smoking, alcohol, psychosocial stress, cohabitant status, education, and ethnicity; * P < 0.01; [§] P < 0.05.*

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Table 7.4 Association between maternal fatty acid concentrations in plasma phospholipids [SD scores and quintiles (Q)] and small-for-gestational-age (SGA) birth[a]

			Univariate model		Multivariate model	
Fatty acid	**n**	**SGA**	Continuous[b]	Categorical[c]	Model 1[c,d]	Model 2[c,e]
		%				
α-Linolenic acid (18:3n-3)			0.86 (0.77, 0.96)[f]*			
Q1	595	15.1		1.29 (0.94, 1.79)	1.03 (0.74, 1.44)	1.04 (0.73, 1.46)
Q2	812	11.6		0.95 (0.69, 1.31)	0.88 (0.63, 1.21)	0.87 (0.63, 1.21)
Q3	661	12.1		1.00	1.00	1.00
Q4	873	10.4		0.85 (0.61, 1.16)	0.85 (0.61, 1.17)	0.84 (0.61, 1.17)
Q5	763	10.0		0.80 (0.58, 1.12)	0.82 (0.58, 1.14)	0.83 (0.59, 1.17)
Eicosatetraenoic acid (20:4n-3)			0.74 (0.66, 0.83)*			
Q1	623	19.9		2.08 (1.54, 2.81)*	1.58 (1.15, 2.17)*	1.50 (1.07, 2.11)[§]
Q2	728	10.3		0.96 (0.69, 1.34)	0.85 (0.61, 1.19)	0.83 (0.59, 1.16)
Q3	787	10.7		1.00	1.00	1.00
Q4	793	10.2		0.95 (0.69, 1.31)	0.98 (0.71, 1.36)	0.98 (0.71, 1.37)
Q5	773	8.7		0.79 (0.57, 1.11)	0.80 (0.57, 1.13)	0.76 (0.54, 1.07)
EPA (20:5n-3)			0.89 (0.79, 0.99)[§]			
Q1	694	16.7		2.04 (1.48, 2.83)*	1.49 (1.05, 2.12)[§]	1.40 (0.97, 2.02)
Q2	762	12.5		1.45 (1.04, 2.03)[§]	1.29 (0.92, 1.82)	1.23 (0.87, 1.74)
Q3	715	9.0		1.00	1.00	1.00
Q4	784	10.1		1.14 (0.81, 1.61)	1.12 (0.79, 1.59)	1.15 (0.81, 1.64)
Q5	749	10.3		1.17 (0.82, 1.65)	1.06 (0.75, 1.51)	1.13 (0.79, 1.62)
DPA (22:5n-3)			0.84 (0.76, 0.94)*			
Q1	724	16.7		1.83 (1.33, 2.50)*	1.50 (1.08, 2.08)[§]	1.49 (1.06, 2.10)[§]
Q2	749	11.7		1.21 (0.87, 1.69)	1.15 (0.82, 1.61)	1.16 (0.83, 1.63)
Q3	717	9.9		1.00	1.00	1.00
Q4	769	9.6		0.97 (0.69, 1.37)	1.05 (0.74, 1.48)	1.06 (0.75, 1.51)
Q5	745	10.3		1.05 (0.75, 1.47)	1.10 (0.78, 1.55)	1.10 (0.78, 1.57)
DHA (22:6n-3)			0.90 (0.82, 1.00)			
Q1	732	13.0		1.30 (0.95, 1.80)	1.20 (0.87, 1.66)	1.10 (0.78, 1.54)
Q2	746	13.4		1.35 (0.99, 1.86)	1.35 (0.98, 1.87)	1.28 (0.92, 1.77)
Q3	740	10.3		1.00	1.00	1.00
Q4	739	11.2		1.11 (0.80, 1.54)	1.16 (0.83, 1.62)	1.17 (0.83, 1.64)
Q5	747	10.3		1.00 (0.72, 1.40)	1.00 (0.71, 1.41)	1.03 (0.73, 1.46)
Linoleic acid (18:2n-6)			1.10 (1.00, 1.21)			
Q1	738	11.5		1.16 (0.83, 1.61)	1.18 (0.85, 1.65)	1.20 (0.85, 1.68)
Q2	740	11.5		1.15 (0.83, 1.60)	1.15 (0.82, 1.60)	1.16 (0.83, 1.63)
Q3	741	10.1		1.00	1.00	1.00
Q4	743	9.7		0.95 (0.68, 1.34)	0.92 (0.65, 1.30)	0.91 (0.64, 1.29)
Q5	742	15.4		1.61 (1.18, 2.20)*	1.42 (1.03, 1.94)[§]	1.34 (0.97, 1.85)

(continued)

Table 7.4 (continued)

			Univariate model		Multivariate model	
Fatty acid	**n**	**SGA**	Continuous[b]	Categorical[c]	Model 1[c,d]	Model 2[c,e]
DGLA (20:3n-6)			0.80 (0.72, 0.89)*			
Q1	737	14.1		1.40 (1.02, 1.91)[§]	1.28 (0.93, 1.76)	1.26 (0.91, 1.74)
Q2	737	14.5		1.45 (1.06, 1.97)[§]	1.42 (1.04, 1.95)[§]	1.38 (1.00, 1.91)[§]
Q3	742	10.5		1.00	1.00	1.00
Q4	740	9.6		0.90 (0.64, 1.27)	0.91 (0.65, 1.28)	0.93 (0.66, 1.31)
Q5	748	9.5		0.89 (0.64, 1.25)	0.90 (0.64, 1.27)	0.86 (0.60, 1.22)
AA (20:4n-6)			1.23 (1.11, 1.35)*			
Q1	740	9.9		0.95 (0.68, 1.34)	0.91 (0.64, 1.28)	0.84 (0.59, 1.19)
Q2	736	10.5		1.02 (0.73, 1.42)	1.04 (0.74, 1.46)	1.03 (0.73, 1.45)
Q3	737	10.3		1.00	1.00	1.00
Q4	747	12.7		1.27 (0.92, 1.75)	1.21 (0.87, 1.67)	1.24 (0.89, 1.73)
Q5	744	14.8		1.51 (1.11, 2.06)[§]	1.30 (0.94, 1.78)	1.24 (0.89, 1.73)
Adrenic acid (22:4n-6)			1.19 (1.09, 1.32)*			
Q1	687	9.6		0.93 (0.65, 1.31)	0.89 (0.63, 1.27)	0.94 (0.66, 1.34)
Q2	690	12.2		1.21 (0.87, 1.68)	1.23 (0.88, 1.72)	1.28 (0.91, 1.80)
Q3	728	10.3		1.00	1.00	1.00
Q4	799	10.8		1.05 (0.76, 1.46)	0.97 (0.70, 1.35)	0.95 (0.68, 1.33)
Q5	800	15.0		1.54 (1.13, 2.09)*	1.34 (0.98, 1.84)	1.23 (0.89, 1.71)
Osbond acid (22:5n-6)			1.12 (1.02, 1.24)[§]			
Q1	722	10.8		0.98 (0.70, 1.37)	1.02 (0.72, 1.43)	1.04 (0.73, 1.46)
Q2	750	12.0		1.10 (0.80, 1.52)	1.16 (0.83, 1.61)	1.15 (0.82, 1.61)
Q3	690	11.0		1.00	1.00	1.00
Q4	758	10.2		0.91 (0.65, 1.28)	0.90 (0.64, 1.27)	0.86 (0.61, 1.21)
Q5	784	14.0		1.32 (0.97, 1.80)	1.18 (0.85, 1.62)	1.08 (0.78, 1.51)
Elaidic acid (18:1n-9*t*)			1.10 (1.00, 1.21)[§]			
Q1	637	11.0		0.95 (0.68, 1.31)	0.80 (0.57, 1.11)	0.81 (0.57, 1.14)
Q2	685	10.7		0.91 (0.66, 1.26)	0.86 (0.62, 1.20)	0.88 (0.63, 1.22)
Q3	814	11.5		1.00	1.00	1.00
Q4	824	11.7		1.01 (0.75, 1.37)	0.99 (0.73, 1.35)	0.93 (0.68, 1.27)
Q5	744	13.2		1.16 (0.86, 1.57)	1.08 (0.79, 1.47)	1.01 (0.74, 1.39)

*[a] Logistic regression analysis with SGA as dependent variable and fatty acid concentration as independent variable. Q1 through Q5 represents the quintile distribution of the relative concentrations as given in Table 7.2. EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid; SGA, birth weight < 10th percentile for gestational age on the basis of sex- and parity-specific standards; OR, odds ratio; [b] Continuous model with the SD score as measure of the fatty acid concentration, OR (95% CI) represents the increase in risk of delivering an SGA infant for each 1-SD increase in fatty acid concentration; [c] Categorical model with quintiles as measure of the fatty acid concentration; OR (95% CI) represents the risk of delivering an SGA infant in the specific quintile compared with the reference quintile (Q3); [d] Adjusted for gestational age at blood sampling, and maternal age and height (note: SGA is already adjusted for gestational age, infant sex, and parity); [e] As in model 1 with additional adjustment for maternal pregravid BMI (linear and quadratic), smoking, alcohol, psychosocial stress, cohabitant status, education, and ethnicity; [f] OR; 95% CI in parentheses (all such values); * P < 0.01; [§] P < 0.05.*

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Table 7.5 Multivariate association between maternal fatty acid profile as represented by the cumulative exposure score and birth weight and small-for-gestational-age (SGA) birth, respectively[a]

		Birth weight		SGA	
Cumulative exposure score[b]	**n**	mean ± SD	*B* ± SE[c]	%	OR (95% CI)[d]
0–1	1716	3569 ± 487	0.0	9.4	1.00
2–3	1192	3510 ± 463	−30.1 ± 15.6	10.0	0.96 (0.74, 1.24)
4–5	519	3408 ± 497	−50.7 ± 22.5*	16.4	1.32 (0.95, 1.83)
≥6	277	3329 ± 469	−124.5 ± 30.3#	23.8	2.12 (1.44, 3.13)#

[a] Linear regression analysis for birth weight as dependent variable and cumulative exposure score as independent variable; logistic regression analysis for SGA as dependent variable and cumulative exposure score as independent variable. SGA, birth weight < 10th percentile for gestational age on the basis of sex- and parity-specific standards; OR, odds ratio.

[b] Based on the univariate associations of the individual fatty acids with birth weight. For each fatty acid positively associated with birth weight (i.e., 18:3n-3, 20:4n-3, 20:5n-3, 22:5n-3, 22:6n-3, and 20:3n-6) the lowest quintile was scored as 1 (exposure); for each fatty acid negatively associated with birth weight (i.e., 18:2n-6, 20:4n-6, 22:4n-6, 22:5n-6, and 18:1n-9t), the highest quintile was scored as 1. After summation, scores were combined into 4 categories (0–1, 2–3, 4–5, and ≥6) and the latter category was defined as the most adverse profile.

[c] Full multivariate model was adjusted for gestational age at blood sampling, gestational age at birth (linear and quadratic), infant sex, maternal age, height, parity, pregravid BMI (linear and quadratic), smoking, alcohol, psychosocial stress, cohabitant status, education, and ethnicity. B ± SE is the unstandardized regression coefficient, representing the difference between the birth weight in the specific category and that in the reference category.

[d] Full multivariate model was adjusted for gestational age at blood sampling, maternal age, height, pregravid BMI (linear and quadratic), smoking, alcohol, psychosocial stress, cohabitant status, education, and ethnicity. OR (95% CI) represents the risk of delivering an SGA infant in the specific category compared with the reference category.

** P < 0.05.*

 # P < 0.01.

Discussion

The results of this large, community-based cohort study suggest that the maternal n-3 and n-6 fatty acid status early in pregnancy affects fetal growth. After adjustment for relevant covariables, both low maternal plasma concentrations of n-3 eicosatetraenoic acid, EPA, and DPA, and of the n-6 LC-PUFA DGLA, and high concentrations of the n-6 LC-PUFA AA were associated with reduced fetal growth, with an estimated 50–60-g decrease in birth weight and 40–50% increase in risk of SGA. The observed negative association between the maternal elaidic acid concentration, the major dietary *trans* fatty acid, and fetal growth disappeared after adjustment. To overcome potential interpretation problems resulting from metabolic interrelations between n-3, n-6, and *trans* fatty acids,[6,30] we developed a measure to define an adverse fatty acid profile. Results showed, for infants of the 7% mothers with the most adverse profile, a growth difference comparable to the estimated effect of smoking,[31] with a 125-g lower birth weight and 100% higher risk of SGA. A lower threshold of adversity, defining just over 20% of the women to have an adverse profile, still corresponded with a 72-g decrease in birth weight and a 50% increase in risk of SGA. Even if we recognize the independent

importance of other physiologic factors, these results, if confirmed, offer perspectives for the dietary prevention of lower birth weight.

Few studies have addressed the association between maternal fatty acid status as measured in blood samples (serum, plasma, or erythrocytes) and birth weight.[8,10-12] In none of the studies significant associations were however reported, possibly by lack of power (n for maternal samples ≤ 582). The remaining observational evidence on the role of *trans*, n-6, and n-3 fatty acids in fetal growth rests on studies addressing either maternal intake[29,32-36] or neonatal status.[9-12,37] Two previous cross-sectional studies investigated the growth-restrictive potential of *trans* fatty acids in term infants,[12,37] with inconsistent results. Our results do not suggest a major influence of *trans* fatty acids on fetal growth, but, because *trans* concentrations in our population were relatively low,[15] we cannot exclude a negative association at higher intakes. The single available cross-sectional study investigating n-6 status of term infants in detail[11] agrees with our findings for AA and DGLA. The apparent opposite effect of DGLA to AA is difficult to comprehend, but it may involve maternal insulin activity. DGLA as well as its n-3 counterpart eicosatetraenoic acid are elongation products of γ-linolenic acid and stearidonic acid, respectively, which are formed in the human body by enzymatic conversion of the parent essential fatty acids linoleic acid (for γ-linolenic acid) and α-linolenic acid (for stearidonic acid). The enzyme involved, δ-6 desaturase, is stimulated by insulin,[38] a hormone also known to influence fetal growth.[39] Alternatively, the AA–DGLA contrast may result from competitive inhibition between AA and DGLA, as suggested for the anti-inflammatory effect of DGLA.[40] Indeed, our effect estimates for DGLA resemble those of the n-3 LC-PUFA EPA, a well-established AA competitor. In comparison with the larger n-3 intake studies, our n-3 LC-PUFA results are in keeping with those of Olsen and Secher[29] and Rogers et al,[36] who reported an ≈80-g reduction in birth weight after adjustment for gestational age, and an (unadjusted) 40–70% increase in intrauterine growth restriction at low n-3 intake.

At this stage, it is difficult to relate our findings to existing randomized clinical trials, even if results appear convergent. So far, no trial in a similar low-to-moderate risk population has commenced supplementation before the 15th week of pregnancy, whereas our results particularly support the possibility of an early effect. In addition, supplements have been restricted to n-3 fatty acids alone and, finally, success or failure may have been influenced by the background fatty acid status.[41] Nevertheless, the established effect of n-3 LC-PUFA supplementation in late gestation on pregnancy duration,[13,14] in combination with our results, marks the relevance of fatty acid supplementation as preventive option at all phases of pregnancy.

Some limitations and strengths of our study should be addressed. Despite the large sample size, our results apply to a relatively healthy sample of pregnant women; our estimates may therefore be too conservative. We were able to measure the maternal fatty acid status in early pregnancy but, given the low-intrusive design, not thereafter. However, our results are not likely to be influenced by changes in fatty acid status in late gestation. Longitudinal

studies have suggested that early pregnancy profile well predicts fatty acid profile later in pregnancy.[24,42] Moreover, our theoretical point of departure was based on existing evidence that the trajectory of fetal growth and development is set at this early stage.[43] A strength of our study is the nonlinear statistical approach, which clearly showed the insufficiency of standard correlational analysis to describe associations. As can be expected from other biological relations,[44] a linear relation between determinant and outcome is an exception rather than rule in a normal population.

The implications of low birth weight for longer-term health and development of children are well established,[1-3] and it has been suggested that, even within the normal range, reduced birth weight may influence a child's cognitive development.[45] Our results, though observational and only indicative of causal relations, suggest that dietary adaptation of the maternal fatty acid profile may help prevent fetal growth restriction, thereby improving later health. Such adaptation may be obtained by supplying additional γ-linolenic acid, EPA, or both, which has been shown to raise concentrations of DGLA and EPA, respectively, without increasing AA.[46] Given the consequences of a lower birth weight for the child both at birth and at later age, a study investigating the feasibility and potential benefits of such intervention is worthwhile.

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References

1. McIntire DD, Bloom SL, Casey BM, Leveno KJ. Birth weight in relation to morbidity and mortality among newborn infants. N Engl J Med 1999;340:1234–8.
2. Barker DJ. Fetal origins of coronary heart disease. BMJ 1995;311:171–4.
3. Osmond C, Barker DJ. Fetal, infant, and childhood growth are predictors of coronary heart disease, diabetes, and hypertension in adult men and women. Environ Health Perspect 2000;108(suppl 3):545–53.
4. Jackson AA, Bhutta ZA, Lumbiganon P. Nutrition as a preventive strategy against adverse pregnancy outcomes. Introduction. J Nutr 2003;133(suppl 2):1589S–91S.
5. Gluckman P, Hanson M. The fetal matrix. Evolution, development and disease. Cambridge: Cambridge University Press, 2005.
6. Innis SM. Perinatal biochemistry and physiology of long-chain polyunsaturated fatty acids. J Pediatr 2003;143(suppl 4):S1–8.
7. Uauy R, Calderon F, Mena P. Essential fatty acids in somatic growth and brain development. World Rev Nutr Diet 2001;89:134–60.
8. Olsen SF, Hansen HS, Secher NJ, Jensen B, Sandstrom B. Gestation length and birth weight in relation to intake of marine n-3 fatty acids. Br J Nutr 1995;73:397–404.
9. Lucas M, Dewailly É, Muckle G, et al. Gestational age and birth weight in relation to n-3 fatty acids among Inuit (Canada). Lipids 2004;39:617–26.
10. Grandjean P, Bjerve KS, Weihe P, Steuerwald U. Birthweight in a fishing community: significance of essential fatty acids and marine food contaminants. Int J Epidemiol 2001;30:1272–8.
11. Rump P, Mensink RP, Kester AD, Hornstra G. Essential fatty acid composition of plasma phospholipids and birth weight: a study in term neonates. Am J Clin Nutr 2001;73:797–806.
12. Elias SL, Innis SM. Infant plasma trans, n-6 and n-3 fatty acids and conjugated linoleic acids are related to maternal plasma fatty acids, length of gestation, and birth weight and length. Am J Clin Nutr 2001;73:807–14.
13. Makrides M, Duley L, Olsen SF. Marine oil, and other prostaglandin precursor, supplementation for pregnancy uncomplicated by pre-eclampsia or intrauterine growth restriction. Cochrane Database Syst Rev 2006;3:CD003402.pub2.
14. 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;83:1337–44.
15. Craig-Schmidt MC. Isomeric fatty acids: evaluating status and implications for maternal and child health. Lipids 2001;36:997–1006.
16. van Eijsden M, van der Wal MF, Bonsel GJ. Folic acid knowledge and use in a multi-ethnic pregnancy cohort: the role of language proficiency. BJOG 2006;113:1446–51.
17. Allison PD. Missing data. Thousand Oaks, CA: Sage Publications, 2001.
18. Radloff LS. The CES-D scale: a self-report depression scale for research in the general population. Applied Psychological Measurement 1977;1:385–401.

19. Spielberger CD, Gorsuch RL, Lushene RE. STAI manual for the State-Trait Anxiety Inventory ("self-evaluation questionnaire"). Palo Alto, CA: Consulting Psychologists Press, 1970.
20. Huizink AC. Mulder EJ, Robles de Medina PG, Visser GH, Buitelaar JK. Is pregnancy anxiety a distinctive syndrome? Early Hum Dev 2004;79:81–91.
21. Crnic KA, Greenberg MT. Minor parenting stresses with young children. Child Dev 1990;61:1628–37.
22. Karasek R, Brisson C, Kawakami N, Houtman I, Bongers I, Amick B. The Job Content Questionnaire (JCQ): an instrument for internationally comparative assessments of psychosocial job characteristics. J Occup Health Psychol 1998;3:322–55.
23. van Eijsden M, van der Wal MF, Hornstra G, Bonsel GJ. Can whole-blood samples be stored over 24 hours without compromising stability of C-reactive protein, retinol, ferritin, folic acid and fatty acids in epidemiologic research? Clin Chem 2005;51:230–2.
24. Al MD, van Houwelingen AC, Kester AD, Hasaart TH, de Jong AE, Hornstra G. Maternal essential fatty acid patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status. Br J Nutr 1995;74:55–68.
25. Otto SJ, van Houwelingen AC, Hornstra G. The effect of different supplements containing docosahexaenoic acid on plasma and erythrocyte fatty acids of healthy non-pregnant women. Nutr Res 2000;20:917–27.
26. Hoving EB, Jansen G, Volmer M, van Doormaal JJ, Muskiet FA. Profiling of plasma cholesterol ester and triglyceride fatty acids as their methyl esters by capillary gas chromatography, preceded by a rapid aminopropyl-silica column chromatographic separation of lipid classes. J Chromatogr 1988;434:395–409.
27. Kaluzny MA, Duncan LA, Merritt MV, Epps DE. Rapid separation of lipid classes in high yield and purity bonded phase columns. J Lipid Res 1985;26:135–40.
28. Morrison WR, Smith LM. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J Lipid Res 1964;5:600–8.
29. Olsen SF, Secher NJ. Low consumption of seafood in early pregnancy as a risk factor for preterm delivery: prospective cohort study. BMJ 2002;324:447–50.
30. Sugano M, Ikeda I. Metabolic interactions between essential and trans-fatty acids. Curr Opin Lipidol 1996;7:38–42.
31. Hammoud AO, Bujold E, Sorokin Y, Schild C, Krapp M, Baumann P. Smoking in pregnancy revisited: findings from a large population-based study. Am J Obstet Gynecol 2005;192:1856–63.
32. Thorsdottir I, Birgisdottir BE, Halldorsdottir S, Geirsson RT. Association of fish and fish liver oil intake in pregnancy with infant size at birth among women of normal weight before pregnancy in a fishing community. Am J Epidemiol 2004;160:460–5.
33. Olsen SF, Olsen J, Frische G. Does fish consumption during pregnancy increase fetal growth? A study of the size of the newborn, placental weight and gestational age in relation to fish consumption during pregnancy. Int J Epidemiol 1990;19:971–7.
34. Olsen SF, Grandjean P, Weihe P, Videro T. Frequency of seafood intake in pregnancy as a determinant of birth weight: evidence for a dose dependent relationship. J Epidemiol Community Health 1993;47:436–40.
35. Oken E, Kleinman KP, Olsen SF, Rich-Edwards JW, Gillman MW. Associations of seafood and elongated n-3 fatty acid intake with fetal growth and length of gestation: results from a US pregnancy cohort. Am J Epidemiol 2004;160:774–83.

36. Rogers I, Emmett P, Ness A, Golding J. Maternal fish intake in late pregnancy and the frequency of low birth weight and intrauterine growth retardation in a cohort of British infants. J Epidemiol Community Health 2004;58:486–92.
37. van Houwelingen AC, Hornstra G. Trans fatty acids in early human development. World Rev Nutr Diet 1994;75:175–8.
38. Brenner RR. Hormonal modulation of Δ6 and Δ5 desaturases: case of diabetes. Prostaglandins Leukot Essent Fatty Acids 2003;68:151–62.
39. Styne DM. Fetal growth. Clin Perinatol 1998;25:917–38.
40. Belch JJ, Hill A. Evening primrose oil and borage oil in rheumatologic conditions. Am J Clin Nutr 2000;71(Suppl 1):352S–6S.
41. Gronn M, Gorbitz C, Christensen E, et al. Dietary n-6 fatty acids inhibit the incorporation of dietary n-3 fatty acids in thrombocyte and serum phospholipids in humans: a controlled dietetic study. Scand J Clin Lab Invest 1991;51:255–63.
42. Otto SJ, van Houwelingen AC, Antal M, et al. Maternal and neonatal essential fatty acid status in phospholipids: an international comparative study. Eur J Clin Nutr 1997;51:232–42.
43. Godfrey KM, Barker DJ. Fetal programming and adult health. Public Health Nutr 2001;4:611–24.
44. Pitsavos CE, Toutouzas PK. Cardiovascular risk factor profile in Greece: results from the CARDIO2000 and ATTICA epidemiological studies. Curr Med Res Opin 2002;18:277–83.
45. Matte TD, Bresnahan M, Begg MD, Susser E. Influence of variation in birth weight within normal range and within sibships on IQ at age 7 years: cohort study. BMJ 2001;323:310–4.
46. Barham JB, Edens MB, Fonteh AN, Johnson MM, Easter L, Chilton FH. Addition of eicosapentaenoic acid to γ-linolenic acid-supplemented diets prevents serum arachidonic accumulation in humans. J Nutr 2000;130:1925–31.

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Chapter 8

General discussion

Introduction

The aim of the present thesis was to elucidate the role of maternal nutrition in explaining ethnicity-related differences in fetal growth, as measured by birth weight at term (≥37.0 weeks' gestation) and prevalence of small-for-gestational-age (SGA) births. The studies described were embedded in the Amsterdam Born Children and their Development (ABCD) study, a prospective community-based cohort study initiated in 2003 by the Municipal Health Service and the Academic Medical Center in Amsterdam. The major aim of the ABCD study is to gain more insight into the association between ethnicity and health, at birth as well as in later life. More specifically, the study aims to elucidate those factors that explain ethnicity-related health disparities, since ethnicity, rather than being a causal factor in itself, must be considered a "basket" factor that incorporates biological, social, and cultural components relevant to health and disease.[1]

In the context of public health policy, Lin and Kelsey[1] give the following arguments for the detailed analysis of ethnicity in relation to health and disease. Such an analysis:

(1) provides leads about etiology;

(2) helps to understand the roles of, and interactions between, genetic and environmental factors;

(3) gives insight into the differences in biology (e.g., disease mechanisms) among ethnic groups;

(4) assesses how the conceptualization of risk factors, symptoms, and disease may differ by ethnic/racial group, so that interventions may be better tailored to specific groups; and

(5) identifies subgroups that may receive unequal prevention screening, or treatment, so that public health programs can be better targeted.

The ultimate goal of the ABCD study is to develop and improve public health programs, i.e., (4) and (5). However, to do this, research into (1), (2), and (3) is required.

In this thesis, we focused on the interrelation between ethnicity, nutrition, and birth weight for two nutrients/nutrient groups in particular: folate/folic acid and the n-3 and n-6 essential polyunsaturated fatty acids. We examined (a) the association between ethnicity and these nutrients (and determinants thereof); (b) the association between ethnicity and birth weight (focusing on explanatory factors other than nutrition); and (c) the role of these nutrients as determinants of birth weight. Data were collected by means of a questionnaire; in addition, a pragmatic approach to blood sampling was applied to measure the maternal nutrient status early in pregnancy. As the validity of these nutrient measurements in blood samples was assumed to depend on the validity of the sampling approach, this was also investigated. More specifically, the following research questions were addressed:

Validity of nutrient analyses:

1. Is a pragmatic approach to blood sampling suitable for valid measurement of nutrient status in a large-scale epidemiologic study? (Chapter 2)

Ethnicity and maternal nutrition:

2. (a) Does periconceptional use of folic acid supplements differ between women from ethnic minority groups and Dutch women; (b) are there ethnic-specific determinants that can explain ethnic differences in folic acid supplement use; and (c) how important is language proficiency as a determinant of use among women who were born in non-Dutch-speaking, non-Western countries? (Chapter 3)

3. (a) How do early pregnancy fatty acid concentrations among ethnic minority women compare to the early pregnancy fatty acid concentrations among Dutch women; and (b) to what extent can fish intake as a source of n-3 long-chain polyunsaturated fatty acids (LC-PUFAs) account for the ethnic variation in maternal n-3 and n-6 LC-PUFA concentrations? (Chapter 4)

Ethnicity and birth weight:

4. (a) How do the term birth weight distributions of ethnic minority women compare to the term birth weight distribution of Dutch women; and (b) to what extent can ethnic differences in birth weight be explained by conventional physiologic and environmental (but non-nutritional) risk factors? (Chapter 5)

Maternal nutrition and birth weight:

5. Is there a role for folate depletion in the association of short interpregnancy intervals with birth weight and SGA risk at term? (Chapter 6)

6. How does the maternal n-3 and n-6 fatty acid status relate to fetal growth as measured by infant birth weight and SGA risk at term? (Chapter 7)

In this chapter we will discuss our results, starting with a reflection on our findings and their implications for research, public health policy, and perinatal care. We will then discuss some general methodological issues and, finally, formulate our conclusions.

Ethnic disparities in birth weight: constitution is important

In Chapter 5, we observed that for the 1st and 2nd generations of all ethnic minority groups the crude birth weight distributions (standardized for gestational age only) were shifted to a lower birth weight (−21 g to −255 g) compared with the Dutch group. Ethnic minority groups

included all of the main ethnic groups in Amsterdam (Surinamese, Antillean, Ghanaian, Turkish, Moroccan) as well as an "other non-Dutch" group. Rather than environmental determinants (most importantly maternal smoking, body mass index (BMI), and work-related stress), constitutional factors (infant sex, maternal parity, age, and height) largely explained these disparities; after adjustment for the latter group of determinants, the birth weights of Turkish, Moroccan, and other non-Dutch newborns were similar to the birth weights of Dutch newborns. The minimal role of modifiable environmental determinants implicates that for these groups the potential for preventive actions – though still important on the individual level – is small.

Although constitutional rather than environmental factors were also responsible for a reduction in the birth weight disparities observed for the groups primarily of African origin (Surinamese, Antilleans, and Ghanaians), these newborns remained significantly smaller than the Dutch newborns (−98 g to −159 g). Explanations for these persisting disparities may include not only nutrition (see next paragraph), but also other unmeasured environmental determinants, constitutional/genetic factors, or their interaction. In this context, a potential pathway worth investigating is the vascular reactivity pathway.[2,3] Women of African origin have shown higher rates of chronic and pregnancy-induced hypertension.[4,5] A genetic predisposition to increased vascular reactivity, in combination with a higher exposure to environmental stress factors (e.g., racism, socioeconomic deprivation),[2] could affect uteroplacental blood flow, and hence, fetal growth.[3] Further research is also required to determine the consequences of the birth weight disparities. At the moment, we do not know if and how the lower birth weights of Surinamese, Antillean and Ghanaian newborns affect their short- or longer-term health. The ABCD study, however, provides a unique opportunity to investigate the longer-term consequences by follow-up measurements in childhood and beyond. With regard to short-term effects, preliminary analysis of weight-specific rates of mortality and morbidity of births registered in the Netherlands Perinatal Registry (PRN) suggests that the smaller size of Surinamese infants at birth is indeed related to more adverse outcomes (G.J. Bonsel, personal communication, 2008).

Role of maternal nutrition in ethnic birth weight disparities

The observed associations of maternal nutrition with both ethnicity (Chapters 3 and 4) and birth weight (Chapters 6 and 7) suggest that maternal nutrition could still – at least in part – explain the observed ethnic disparities in birth weight. This suggestion also emerges from **Table 8.1**, which illustrates the role of the maternal fatty acid profile in explaining the birth weight differences between ethnic groups; note, however, that the changes (model 2 vs. model 1) are modest. Further studies to explore the impact of other nutritional components would be worthwhile, but, as our studies show, these too should take into account the complex nature of

the interrelation between ethnicity, nutrition, and birth weight. More specifically, they should consider (1) the nonlinearity of the association between nutrition and birth weight; (2) the intercorrelations between nutrients; and (3) the indirect relationship between nutrient intake and nutrient status in maternal blood.

Table 8.1 Differences in birth weight (standardized for gestational age, in grams) between the ethnic groups, adjusted for constitutional and environmental non-nutritional determinants (model 1) and fatty acid profile (model 2)[a,b]

			Model 1		Model 2	
		n	*B*	95% CI	*B*	95% CI
Ethnicity						
Dutch (reference)		2237	0.0		0.0	
Surinamese	1st generation	146	−140.9	−214.6, −67.2	−112.8	−187.5, −38.0
	2nd generation	91	−169.0	−260.0, −77.9	−150.3	−241.6, −58.9
Antillean	1st generation	41	−104.4	−233.1, 24.3	−88.2	−216.8, 40.4
	2nd generation	15	−65.9	−274.6, 142.9	−64.7	−273.0, 143.6
Turkish	1st generation	120	35.5	−47.0, 118.0	75.9	−8.6, 160.4
	2nd generation	34	108.0	−35.9, 251.8	149.4	4.5, 294.4
Moroccan	1st generation	182	−20.9	−90.7, 48.9	−2.8	−73.0, 67.4
	2nd generation	42	−34.1	−163.6, 95.3	−13.0	−142.6, 116.6
Ghanaian	1st + 2nd generation	37	−106.8	−244.7, 31.1	−118.9	−256.7, 18.8
Other	1st generation	554	28.8	−13.3, 70.9	38.2	−4.0, 80.5
	2nd generation	167	0.2	−64.4, 64.9	3.6	−61.0, 68.1
Fatty acids – cumulative exposure score[b]						
0–1		1707			0.0	
2–3		1181			−31.8	−62.7, −0.9
4–5		511			−57.3	−102.0, −12.7
≥6		267			−133.9	−195.0, −72.8

[a] Linear regression analysis, B (95% CI) is the unstandardized regression coefficient with 95% confidence interval, representing the difference in the birth weight in the specific ethnic group and that in the Dutch reference group. Model 1: adjusted for infant sex, parity, maternal age, height, education, cohabitant status, BMI, smoking, alcohol consumption, depression, and work stress (see Chapter 5). Model 2: as model 1, plus additional adjustment for maternal fatty acid profile.

[b] The maternal fatty acid profile is defined by the cumulative exposure score. The exposure score is based on the univariate associations of the individual fatty acids with birth weight (see Chapter 7). For each fatty acid positively associated with birth weight (i.e., 18:3n-3, 20:4n-3, 20:5n-3, 22:5n-3, 22:6n-3, and 20:3n-6), the lowest quintile was scored as 1 (exposure); for each fatty acid negatively associated with birth weight (i.e., 18:2n-6, 20:4n-6, 22:4n-6, 22:5n-6, and 18:1n-9t), the highest quintile was scored as 1. After summation, scores were combined into 4 categories (0–1, 2–3, 4–5, and ≥6), and the latter category was defined as the most adverse profile.

Nonlinear association

As shown in Chapters 6 and 7, the association between a specific nutritional factor and birth weight is likely to be nonlinear. As also noted by others,[6] it is more probable that a relationship between (a) a nutrient and (b) functions dependent on that nutrient will plateau at levels above need. For example, Figure 6.1 in Chapter 6 illustrates that the positive effect of the use of supplements containing folic acid on fetal growth (as reflected by a higher mean birth weight and lower SGA risk in comparison to nonusers) is present at short (typically defined as <6 months) intervals only, i.e., among the women most at risk of folate depletion.[7] This positive effect of folic acid supplement use is further illustrated in **Table 8.2**, which shows the mean birth weight and SGA prevalence according to supplement use in intervals <6 months compared to intervals of 18 to 23 months.

Similarly, in Chapter 7 we observed that the specific associations of the n-3 and n-6 fatty acids with birth weight were present at the extremes only. Low concentrations of n-3 eicosatetraenoic acid (20:4n-3), eicosapentaenoic acid (EPA, 20:5n-3) and docosapentaenoic acid (DPA, 22:5n-3) as well as the n-6 LC-PUFA dihomo-γ-linolenic acid (DGLA, 20:3n-6) and high concentrations of the n-6 LC-PUFA arachidonic acid (AA, 20:4n-6) affected fetal growth, with an estimated 50 to 60 g decrease in birth weight and 40% to 50% increase in SGA risk at the extremes (lower/upper quintile) when compared to average concentrations (middle quintile). Thus, when investigating the role of nutritional factors in ethnic birth weight disparities, the nonlinearity of the associations should be considered, since simple linear correlation statistics will not be sufficient in that case and can be misleading.

Table 8.2 Birth weight and small-for-gestational-age (SGA) prevalence for term births among nonusers, late users, and early users of folic acid supplements: comparison between mothers with interpregnancy intervals of <6 months and mothers with interpregnancy intervals of 18 to 23 months

	Interval <6 months			**Interval 18–23 months**		
	Nonusers	Late users	Early users	Nonusers	Late users	Early users
n	45	44	34	96	137	174
Birth weight (g)	3281 ± 404[a]	3510 ± 491	3636 ± 543	3633 ± 605	3704 ± 500	3637 ± 453
SGA (%)	35.6	20.5	8.8	13.5	11.7	10.3

[a] *Mean ± SD (all such values).*

Nutrient intercorrelations

Aside from the "single nutrients" such as folate (nutrients with an established role in pregnancy at levels not normally obtainable from foods),[8] the required physiologic amounts of the nutrients essential to fetal growth are generally obtained from the pregnant woman's diet. However, diet represents a complex set of exposures that can be strongly intercorrelated,

an issue which studies of single nutrients in isolation – though important first steps in establishing nutritional effects – do not take into account.[9,10] In this thesis, the importance of intercorrelations between nutrients was demonstrated in Chapter 7, in which we examined not only the individual associations of the maternal n-3, n-6, and *trans* fatty acids with birth weight, but also the role of the overall maternal fatty acid profile. This measure, defined by the combination of the individual fatty acid concentrations, was specifically developed to consider the metabolic interrelations between these fatty acids. The analysis showed that infants born to the 7% of mothers with the most adverse profile were 125 g lighter and twice as likely to be SGA, an effect comparable to that of smoking.[11] These results, in conjunction with the emerging evidence on the importance of a healthy diet (which would indicate an adequate overall nutrient status),[10,12,13] stress the importance of more detailed research into the significance of nutrient intercorrelations for fetal growth, as has also been argued by others.[9,14,15] In this context, additional research is recommended to investigate in particular the synergy between folate and the n-3 LC-PUFAs that has recently been suggested by some studies,[16,17] but contradicted by others.[18-20] Since nutrient status was extensively measured in the ABCD study (in addition to folate and the n-3 and n-6 fatty acids, we measured the minerals iron, zinc, magnesium, and calcium, and the vitamins A, D, and B1), future studies are also planned to examine these nutrients, their interrelations, and their associations with ethnicity and birth weight.

Indirect relationship between nutrient intake and status

Much of our knowledge of the role of maternal nutrition in fetal growth has been derived from observational studies and intervention trials in which low or high maternal intakes have been associated with adverse or favorable pregnancy outcomes.[8] However, the final supply of nutrients to the placenta not only depends on the mother's intake, but also on her intermediary metabolism and endocrine status, her partitioning of nutrients among storage, use, and circulation, and cardiovascular adaptations that enhance uterine blood flow.[14] Consequently, what is measured at the intake level may not necessarily correspond to what is measured at a more physiologic level, i.e., in maternal blood. This discrepancy was especially demonstrated in Chapter 4, in which we described the interethnic differences in maternal n-3 and n-6 fatty acid concentrations and examined the role of fish and fish oil consumption as an explanation for the observed differences in concentrations of EPA and docosahexaenoic acid (DHA, 22:6n-3), as well as DGLA and AA. Compared to Dutch women, Surinamese, Antillean, Turkish, and Moroccan women had generally lower concentrations of n-3 fatty acids but higher concentrations of n-6 fatty acids, with the exception of DGLA. Ghanaian women, in contrast, had higher concentrations of the n-3 fatty acids EPA, DPA, and DHA, but generally lower concentrations of n-6 fatty acids. Interestingly, while differences were most pronounced in the groups who reported the lowest and highest fish intake (Turkish and Ghanaian, respectively), the explanatory strength of this factor was only minor. Thus,

rather than intake variation, the observed differences assumingly reflect metabolic variation between ethnic groups.

Usually, in large epidemiologic (cohort) studies a food frequency questionnaire (FFQ) is preferred over other assessments of the maternal diet or nutrient status, being a convenient and inexpensive method to measure hundreds or even thousands of people.[21] However, as our results show, the use of biomarkers can be a valuable addition to gain a better understanding of the physiologic aspects of nutrition and their relevance to health and disease as well as any related ethnic disparities. That a valid measurement of biomarkers does not necessarily require central collection and immediate processing, as is generally assumed, was demonstrated in Chapter 2. Here we observed that a pragmatic approach to blood collection (i.e., a decentralized collection with samples sent by mail or courier to a central laboratory) still allowed for a valid analysis of the biomarkers of interest: most markers showed limited variance within a 96-h storage period, and although for folate, linoleic acid, and adrenic acid significant changes (≥10%) over time were observed, these changes did not significantly affect measures of 28h-validity (intraclass correlation coefficients ≥ 0.9, n = 50 bootstrap Spearman rank correlation coefficients ≥ 0.8).

Arguably, in some cases the FFQ approach may still be preferred over biomarker measurements, depending also on the relative validity of the questionnaire. Studies that validated self-reported folic acid supplement use by comparison with biomarker measurements have reported strong and significant linear associations between folate concentrations and folic acid intake,[22,23] suggesting that when women report taking more folic acid, and for a longer period of time, their serum and red blood cell levels increase correspondingly. Since similar observations were done within the ABCD study (where serum folate concentrations among users was generally twice that of concentrations among nonusers)[24] and we aimed for groups of a sufficient size for analysis, we preferred to include supplement use rather than blood measurements of folate in Chapter 6.

Improving maternal nutrition

When considering the implications of our results for public health policy and perinatal care, a distinction must be made between the n-3 and n-6 essential polyunsaturated fatty acids and folate.

As noted before, folate is a nutrient that already has an established effect in pregnancy already, at intake levels unattainable by diet alone.[8] An adequate folate status in pregnancy is undisputedly important for preventing neural tube defects, and may be relevant for preventing not only lower birth weight, but also other pregnancy complications such as preeclampsia, preterm

birth, stillbirth, and abortion.[25] Public health programs to promote the intake of folic acid supplements (ensuring an adequate status) have been implemented since the 1990s,[25,26] but have met with limited success among ethnic minority groups.[27,28] In our study (Chapter 3) we also observed folic acid supplement use to be significantly lower among women born in non-Dutch-speaking, non-Western countries (<41%) than among women born in the Netherlands (86%) or in another Western country (78%). While the use of folic acid supplements was primarily determined by knowledge about folic acid, the key factor for this knowledge, in both the Western and non-Western groups, was language proficiency.

Perhaps the best alternative to the current supplementation policy in the Netherlands would be a fortification policy. However, fortification has been debated, on the one hand with regard to the short and longer term benefits and risks, on the other hand with regard to "the right of personal integrity".[29] Although this debate recently received a new impetus,[30] it seems unlikely that fortification will be implemented in the near future, and other approaches to enhance folic acid intake among ethnic minority groups should be considered. From a more general perspective, the language education recently made mandatory by the Dutch government for all new immigrants is a promising approach, particularly if it integrates information about prenatal health and health care. Alternatively, the ethnic health advisors appointed some years ago in maternal and child health centers throughout Amsterdam, can help target the groups most at risk. Within their own cultural context and in their own language, these advisors can educate women about folic acid use as well as family planning, the latter being an important strategy to avoid folate depletion associated with short interpregnancy intervals.

At this stage, no evidence-based intervention strategies exist to improve the maternal n-3 and n-6 essential fatty acid status, although in the general population the use of fish oil supplements as a source of the n-3 LC-PUFAs seems to have increased over the past few years. While overall our results suggest that research into the effects of either a dietary or pharmacological adaptation of the maternal n-3 and n-6 fatty acid intake would be worthwhile, some circumspection is necessary. Dietary change in a multi-ethnic society could be difficult to achieve, because it requires an intensive measure (e.g., dietary counseling)[31] that must take account of existing cultural patterns and trends and recognize the heterogeneity between as well as within ethnic groups.[32] In terms of feasibility, perhaps more can be expected from a pharmacological adaptation, i.e., via supplementation. Still, for both strategies, the expectations of intervention effects should not be exaggerated, given the dynamics of fatty acid metabolism (see Chapter 4). Monitoring of the effect should thus include measurement of nutrient status before and after the intervention.

Methodological considerations

As does any epidemiologic study, the ABCD study has its methodological strengths and limitations. The methodological considerations directly relevant to the specific studies have been discussed in the *Discussion* sections of the previous chapters. In this section, we will address some issues of general importance: (1) the definition of ethnicity, (2) selective nonresponse, and the strengths and limitations of (3) exposure and (4) outcome measurements.

Defining ethnicity

In epidemiologic and public health research, the concept of ethnicity continues to be a source of debate.[33] As a multidimensional concept, ethnicity is not well-defined, and often encompasses different classifications, depending on the context in which the definition is made. Broadly, three dimensions of ethnicity may be distinguished, all three of which appear relevant to perinatal health: (1) race/genetic constitution; (2) sociocultural orientation; and (3) migrant status. These dimensions are often approached from a cross-sectional perspective, whereas all three are to some extent dynamic, through genetic admixture, acculturation, and adaptation to a host country. Measurement is often a challenge, both in terms of classification (which categories make up the classification?) and identification (on what basis do you identify a person as belonging to a certain category?). For example, whereas the classification of race is often based on geographical origin (or ancestry),[34] identification usually relies on records of one's physical appearance (e.g., skin color) by the researcher or care provider, which is a subjective and imprecise measure.[35] With respect to sociocultural orientation, it is interesting to note that the concept itself includes several dimensions and may consequently be measured very differently depending on the underlying research question. Among the measurement strategies most relevant, those incorporating country of birth may be considered most stable; both cultural characteristics and self-identification can be quite fluid and change over time.[1,36] Also the concept of migrant status may encompass several dimensions that could be difficult to capture, such as the reason for migration, the experience of migration itself, and orientation towards return migration.[36]

In the ABCD study, we aimed to capture all three dimensions; in our view, the classification most suitable for this was country of birth. As a measure of geographical origin it reflects the genetic heritage,[34,37] as well as the social heritage of culture and traditions that are inherent to the concept of ethnicity.[33] In addition, by including maternal country of birth, this approach also allowed for the distinction between women who themselves immigrated (1st generation) and women who are the children of immigrants (2nd generation). Useful as the classification based on country of birth may be, it should be kept in mind that this measure does not capture heterogeneity in history, culture, language, or dietary preferences within ethnic groups,[1] as exists, for example, among the Creole and Hindustani populations of Surinam.[36] However, to the extent that information was available that allowed us to distinguish between these groups,

the Surinamese-Creole and Surinamese-Hindustani people did not differ from each other in outcomes (see Chapter 5). Moreover, it can be assumed that in the Amsterdam population, the Surinamese-Creole group largely outnumbers the Surinamese-Hindustani group.[38]

Selective nonresponse

The overall response rate of the ABCD study was 67%, which agrees with the response rates observed in other large-scale community-based pregnancy cohorts, such as the Generation R study in the Netherlands (61%)[39] and the Southampton Women's Survey (75%)[40] and ALSPAC study (85%) in the UK.[41] During data collection, two supportive measures were taken to enhance enrolment of foreign-born women in particular: we (1) provided a Turkish, Arabic, or English translation to women born in Turkey, Morocco, or another non-Dutch-speaking country, respectively, and (2) offered of oral administration to women who were illiterate or had reading difficulties. Overall, these measures resulted in an adequate response among foreign-born women, although response remained lower than among Dutch-born women: 42% to 64% vs. 77% for the questionnaire, 31 to 55% vs. 59% for the biomarker study. To investigate the degree of selection bias resulting from this selective ethnic nonresponse, a nonresponse analysis by anonymous linkage with the PRN was conducted. The results confirmed the selective ethnic nonresponse with lower participation rates among women from non-Western ethnic origin, but indicated that selection bias was minimal: the association between ethnicity and low birth weight was similar in both the response and nonresponse group.[42] This suggests that, with our study population, conclusions about the associations between ethnicity, birth weight determinants, and birth weight can validly be drawn.

Exposure measurement

The measurement of nutrient status in conjunction with the questionnaire allowed us to capture a large set of risk factors relevant to fetal growth, but we are aware that this advantage may have been counterbalanced by the questionnaire's self-reporting nature. However, given the prospective design of the study, and the assurance of confidentiality provided to all women involved, we expect any information bias to be minimal. All exposures/risk factors were, by design, measured as early in pregnancy as possible, and it could be argued that our results are influenced by changes in exposures occurring in late pregnancy. However, our focus on early pregnancy was chosen on the basis of the existing evidence that the trajectory of fetal growth and development is set at this early stage[43] and under the assumption that any changes in risk factors are most likely attenuations (e.g., women with the most stressful working conditions taking earlier leave).[44] Nevertheless, as variability in nutrient status in particular cannot be excluded, future research into the late effects of nutrients is recommended.

Arguably, the definition of early pregnancy can be arbitrary. All participants in the ABCD study were approached during their first antenatal visit to their obstetric care provider; and variation in the timing of this appointment unavoidably introduced variation in timing of

the questionnaire (and biomarker measurements), with the largest delays observed for women of ethnic minority background.[45] Still, the majority of women (90%) filled out their questionnaires in the first half of pregnancy (≤20th week), and 60% before the 16th week, which in our view justifies the reference to early pregnancy for all women. It should be noted that the timing and nature of the questionnaire may have also resulted in a suboptimal measurement of medical factors like chronic or gestational hypertension and diabetes, which are particularly relevant to placental function and hence to nutrient supply to the fetus. With the recent linkage of ABCD records to information from the PRN,[42] more reliable information has become available, which can be included in future analyses.

Outcome measurement

In this thesis we used term birth weight (as a continuous measure) and SGA (dichotomized) as indicators of fetal growth. Ideally, the assessment of the determinants of fetal growth would start from the expected trajectory for each infant throughout the course of pregnancy, and quantify the deviation in actual growth relative to the expected pattern.[46] However, for most studies, including ours, birth weight is often the only feasible measure. To the extent that the trajectory of fetal growth has been measured in a pregnancy cohort, analyses have not yet provided new information on the determinants of growth or explanations of ethnic disparities.[47,48]

It should be noted that for the classification of SGA, an accurate assessment of not only birth weight but also gestational age is necessary.[49] In the ABCD study, information on birth weight and gestational age as recorded by the obstetric care providers was obtained via the Youth Health Care department of the Municipal Health Service. Since in the Netherlands a routine ultrasound measurement is offered to all pregnant women starting obstetric care and is accepted by the majority of women (>90%), we believe accurate pregnancy dating was ascertained.

In conclusion

In this thesis, we aimed to elucidate the role of maternal nutrition in explaining ethnic disparities in birth weight. From the studies described, the following three conclusions can be drawn.

First, whereas nutritional factors were shown to be relevant to fetal growth, their role in explaining ethnic disparities in birth weight appears to be modest. Although preventing inadequate maternal nutrition is important at an individual level, caution is advised with regard to expectations of intervention effects at the population level. With respect to the n-3 and n-6 essential polyunsaturated fatty acids, interventions aimed at improving the maternal

profile of these fatty acids still require further study, which should take into account the dynamics of fatty acid metabolism. Improving the maternal periconceptional folate status, still of undisputed importance for preventing neural tube defects, may in a multi-ethnic society best be achieved by fortification, but this option is still being debated in the Netherlands. Promising alternative strategies are the employment of ethnic health advisors in maternal and child health care centers and language courses for immigrants: both measures allow for the dissemination of linguistically appropriate, comprehensive information about folic acid use as well as family planning, the latter being an important measure to prevent folate depletion in a subsequent pregnancy.

Second, when examining ethnic disparities in health and disease, three concepts of ethnicity are relevant: (1) race/genetic constitution; (2) sociocultural orientation; and (3) migrant status. Which concepts to consider depends on their relative importance to the health problem and research question at stake. For example, research into the role of nutrition in explaining ethnic disparities in perinatal health requires an understanding of the dietary (sociocultural) as well as metabolic (genetic) aspects of nutrition. The same is true when deciding on a public health strategy. Some problems (e.g., lower birth weight) may require a more tailored intervention restricted to specific subgroups (women of African descent), whereas other problems (folic acid supplement use) may benefit best from a universal measure (language education for all immigrants).

Third, it can be assumed that the observed ethnic disparities, both in birth weight and the determinants thereof, are also relevant to ethnic disparities in later life (the "fetal origins of disease" hypothesis). In time, the ABCD study will provide more insight into the consequences of an adverse fetal environment to health and disease in childhood, and into the extent to which ethnic health differences are indeed explained by ethnic differences in the fetal environment. As such, the study will form a basis for developing and implementing early life interventions.

References

1. Lin SS, Kelsey JL. Use of race and ethnicity in epidemiologic research: concepts, methodological issues, and suggestions for research. Epidemiol Rev 2000;22:187–202.
2. Fiscella K. Races, genes and preterm delivery. J Natl Med Assoc 2005;97:1516–26.
3. Fiscella K. Racial disparity in infant and maternal mortality: confluence of infection, and microvascular dysfunction. Matern Child Health J 2004;8:45–54.
4. Kurian AK, Cardarelli KM. Racial and ethnic differences in cardiovascular disease risk factors: a systematic review. Ethn Dis 2007;17:143–52.
5. Fang J, Madhaven S, Alderman MH. The influence of maternal hypertension on low birth weight: differences among ethnic populations. Ethn Dis 1999;9:369–76.
6. Innis SM. Fatty acids and early human development. Early Hum Dev 2007;83:761–6.
7. Smits LJ, Essed GG. Short interpregnancy intervals and unfavourable pregnancy outcome: role of folate depletion. Lancet 2001;358:2074–7.
8. Picciano MF. Pregnancy and lactation: physiological adjustment, nutritional requirements and the role of dietary supplements. J Nutr 2003;133:1997S–2002S.
9. Jackson AA, Robinson SM. Dietary guidelines for pregnancy: a review of current evidence. Public Health Nutr 2001;4:625–30.
10. Knudsen VK, Orozova-Bekkevold IM, Mikkelsen TB, Wolff S, Olsen SF. Major dietary patterns in pregnancy and fetal growth. Eur J Clin Nutr 2007;doi:10.1038/sj.ejcn.1602745.
11. Hammoud AO, Bujold E, Sorokin Y, Schild C, Krapp M, Baumann P. Smoking in pregnancy revisited: findings from a large population-based study. Am J Obstet Gynecol 2005;192:1856–63.
12. Mikkelsen TB, Osler M, Orozova-Bekkevold I, Knudsen VK, Olsen SF. Association between fruit and vegetable consumption and birth weight: a prospective study among 43,585 Danish women. Scand J Public Health 2006;34:616–22.
13. Mitchell EA, Robinson E, Clark PM, et al. Maternal nutritional risk factors for small for gestational age babies in a developed country: a case-control study. Arch Dis Child Fetal Neonatal Ed 2004;89:F431–5.
14. Fall CH, Yajnik CS, Rao S, Davies AA, Brown N, Farrant HJ. Micronutrients and fetal growth. J Nutr 2003;133(suppl 2):1747S–56S.
15. Keen CL, Clegg MS, Hanna LA, et al. The plausibility of micronutrient deficiencies being a significant contributing factor to the occurrence of pregnancy complications. J Nutr 2003;133(suppl 2):1597S–605S.
16. Krauss-Etschmann S, Shadid R, Campoy C, et al. Effects of fish-oil and folate supplementation of pregnant women on maternal and fetal plasma concentrations of docosahexaenoic acid and eicosapentaenoic acid: a European randomized multicenter trial. Am J Clin Nutr 2007;85:1392–400.
17. Umhau JC, Dauphinais KM, Patel SH, et al. The relationship between folate and docosahexaenoic acid in men. Eur J Clin Nutr 2006; 60:352–7.
18. Dullemeijer C, Durga J, Brouwer IA, Verhoef P. Erythrocyte folate and plasma DHA in the FACIT study. Lancet 2007;370:216.

19. Crowe FL, Skeaff CM, McMahon JA, Williams SM, Green TJ. Lowering plasma homocysteine concentrations of older men and women with folate, vitamin B-12, and vitamin B-6 does not affect the proportion of (n-3) long chain polyunsaturated fatty acids in plasma phosphatidylcholine. J Nutr 2008;138:551–5.
20. Assies J, Lok A, Bockting CL, et al. Fatty acids and homocysteine levels in patients with recurrent depression: an explorative pilot study. Prostaglandins Leukot Essent Fatty Acids 2004;70:349–56.
21. Schatzkin A, Kipnis V, Carroll RJ, et al. A comparison of a food frequency questionnaire with a 24-hour recall for use in an epidemiological cohort study: results from the biomarker-based Observing Protein and Energy Nutrition (OPEN) study. Int J Epidemiol 2003;32:1054–62.
22. Burton A, Wilson S, Gillies AJ. Folic acid: is self reported use of supplements accurate? J Epidemiol Community Health 2001;55:841–2.
23. Brantsæter AL, Haugen M, Hagve TA, et al. Self-reported dietary supplement use is confirmed by biological markers in the Norwegian Mother and Child Cohort Study (MoBa). Ann Nutr Metab 2007;51:146–54.
24. Jansen E, van der Wal M, van Eijsden M, Bonsel G. Foliumzuurinname van zwangeren nog te laag (Folic acid supplement use among pregnant women remains low). Voeding Nu 2006;9:18–20 (in Dutch).
25. Tamura T, Picciano MF. Folate and human reproduction. Am J Clin Nutr 2006;83:993–1016.
26. Cornel MC, de Smit DJ, de Jong-van den Berg LT. Folic acid – the scientific debate as a base for public health policy. Reprod Toxicol 2005;20:411–5.
27. Howell SR, Barnett AG, Underwood MR. The use of pre-conceptional folic acid as an indicator of uptake of a health message amongst white and Bangladeshi women in Tower Hamlets, east London. Fam Pract 2001;18:300–3.
28. Bakker MK, Cornel MC, de Walle HE. Kennis over en gebruik van periconceptioneel foliumzuur onder allochtone en westerse vrouwen, na de publiekscampagne in 1995 (Awareness and periconceptional use of folic acid among non-western and western women in the Netherlands following the 1995 publicity campaign). Ned Tijdschr Geneeskd 2003;147:2426–30 (in Dutch).
29. Cornel M. Tien jaar na de foliumzuurcampagne: bescheiden succes of gemiste kans? (Ten years after the folic acid campaign: a small success or a missed chance?). TSG tijdschrift voor gezondheidswetenschappen 2005;83:391–2 (in Dutch).
30. Gezondheidsraad. Naar een optimaal gebruik van foliumzuur (Towards an optimal use of folic acid). Den Haag: Gezondheidsraad, 2008 (in Dutch).
31. Piirainen T, Isolauri E, Lagström H, Laitinen K. Impact of dietary counselling on nutrient intake during pregnancy: a prospective cohort study. Br J Nutr 2006;96:1095–104.
32. Thomas J. Nutrition intervention in ethnic minority groups. Proc Nutr Soc 2002;61:559–67.
33. Bhopal R. Glossary of terms relating to ethnicity and race: for reflection and debate. J Epidemiol Community Health 2004;58:441–5.
34. Risch N, Burchard E, Ziv E, Tang H. Categorization of humans in biomedical research: genes, race and disease. Genome Biol 2002;3(7):comment 2007.
35. Senior PA, Bhopal R. Ethnicity as a variable in epidemiological research. BMJ 1994;309:327–30.
36. Stronks K, Kulu Glasgow I, Klazinga N. The identification of ethnic groups in health research, additional to the country of birth classification. Amsterdam: Academic Medical Center, 2004.

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37. Burchard EG, Ziv E, Coyle N, et al. The importance of race and ethnic background in biomedical research and clinical practice. New Engl J Med 2003; 348:1170–5.
38. Choenni C, Harmsen C. Geboorteplaats en etnische samenstelling van Surinamers in Nederland. (Place of birth and ethnic composition of Surinamese in the Netherlands). Bevolkingstrends 2007;55(1):74–8 (in Dutch).
39. Jaddoe VW, Mackenbach J P, Moll HA, et al. The Generation R Study: design and cohort profile. Eur J Epidemiol 2006;21:475–84.
40. Inskip HM, Godfrey KM, Robinson SM, et al. Cohort profile: the Southampton Women's Survey. Int J Epidemiol 2006;35:42–8.
41. Golding J, ALSPAC Study Team. The Avon Longitudinal Study of Parents and Children (ALSPAC) – study design and collaborative opportunities. Eur J Endocrinol 2004;151(suppl 3):U119–23.
42. Tromp M, van Eijsden M, Ravelli AC, Bonsel GJ. Anonymous non-response analysis in the ABCD cohort study enabled by probabilistic record linkage (submitted 2007).
43. Godfrey KM, Barker DJ. Fetal programming and adult health. Public Health Nutr 2001;4:611–24.
44. Croteau A, Marcoux S, Brisson C. Work activity in pregnancy, preventive measures, and the risk of delivering a small-for-gestational-age infant. Am J Public Health 2006;96:846–55.
45. Alderliesten ME, Vrijkotte TG, van der Wal MF, Bonsel GJ. Late start of antenatal care among ethnic minorities in a large cohort of pregnant women. BJOG 2007;114:1232–9.
46. Savitz DA, Hertz-Picciotto I, Poole C, Olshan AF. Epidemiologic measures of the course and outcome of pregnancy. Epidemiol Rev 2002;24:91–101.
47. Drooger JC, Troe JW, Borsboom GJ, et al. Ethnic differences in prenatal growth and the association with maternal and fetal characteristics. Ultrasound Obstet Gynecol 2005;26:115–22.
48. Jaddoe VW, Verburg BO, de Ridder MA, et al. Maternal smoking and fetal growth characteristics in different periods of pregnancy. The Generation R Study. Am J Epidemiol 2007;165:1207–15.
49. Ergaz Z, Avgil M, Ornoy A. Intrauterine growth restriction – etiology and consequences: what do we know about the human situation and experimental animal models? Reprod Toxicol 2005;20:301–22.

Summary
Samenvatting

Summary

The relevance of birth weight, not only as an outcome measure (reflecting health in pregnancy), but also as an indicator of children's future health ("fetal origins of disease" hypothesis), is well accepted. Worldwide, large differences exist in the birth weight distribution of ethnically diverse populations, with the lowest birth weights and highest proportion of growth-restricted infants usually found among minority populations. From a preventive point of view a better understanding of the factors that influence fetal growth, particularly if this influence is negative and related to longer-term adverse outcomes, is important in order to explain and address these ethnic disparities. Interestingly, the birth weight disparities often coincide with differences in maternal nutritional adequacy, which raises the question: are differences in maternal nutrition the key to explaining ethnicity-related differences in birth weight?

The aim of the present thesis, as described in **Chapter 1**, was to elucidate the role of maternal nutrition in explaining ethnicity-related differences in fetal growth, as measured by birth weight at term (≥37.0 weeks gestation) and prevalence of small-for-gestational-age (SGA) births. For this purpose, we examined (1) the association between ethnicity and maternal nutrition (and determinants thereof); (2) the association between ethnicity and birth weight (focusing on explanatory factors other than nutrition); and (3) the role of maternal nutrition as a determinant of birth weight. The focus was on two specific nutrients/nutrient groups: folate/folic acid and the n-3 and n-6 essential fatty acids and their derivatives (the long-chain polyunsaturated fatty acids, LC-PUFAs).

All studies were embedded in the Amsterdam Born Children and their Development (ABCD) study, a large, prospective cohort study in Amsterdam, the Netherlands. Between January 2003 and March 2004, 12 373 pregnant women were approached to participate in this study during their first prenatal visit to an obstetric care provider (general practitioner, midwife, or hospital gynecologist). In total, 8266 women (67%) agreed to participate and filled out an extensive questionnaire, covering nutrition (folic acid supplement use and fish consumption) as well as sociodemographic data, obstetric history, lifestyle, and psychosocial factors. In the study population, all of the main ethnic groups in Amsterdam were represented: Dutch, Surinamese, Antillean (including Aruba), Turkish, Moroccan, and Ghanaian (country of birth definition). Fifty-three percent of the respondents additionally participated in the biomarker study, in which maternal nutrient status was measured in blood. For this, women were invited to donate an extra blood sample during the routine blood collection for screening purposes, after which samples were sent to the Regional Laboratory of Amsterdam for processing and storage until analysis.

Since we assumed the validity of the nutrient measurements in blood samples to depend on the validity of this sampling approach, we started our research with a pilot study investigating the

appropriateness of our pragmatic approach of blood collection (**Chapter 2**). Blood samples of 41 female volunteers (n = 20 for serum, n = 21 for plasma) were collected and stored in the dark at 21 °C until processing. One sample was processed within 2 h after sampling (baseline), the remaining samples were processed 2, 4, 24, 26, 28, and 96 hours after baseline. Results showed that during 96 h of storage, concentrations of CRP, ferritin, retinol, all n-3 fatty acids, and most n-6 fatty acids changed less than 10%. Concentrations of folate and linoleic acid (18:2n-6) significantly decreased (16.5% and 14.0% respectively), while the concentration of adrenic acid (22:4n-6) significantly increased (16.2%). However, for none of these biomarkers we observed a relevant influence of the time-related changes on the 28-h reliability or validity coefficients (28 h being the maximum delay time allowed in the ABCD study): intraclass correlation coefficients were ≥0.9, Spearman rank correlation coefficients were ≥0.8 (n = 50 bootstrap sample). This indicates that, in samples stored for a maximum of 28 h, the validity of standard epidemiological analyses will not be compromised.

Subsequently, in Chapters 3 and 4, we focused on the ethnic heterogeneity in nutrient intake and nutrient status. In **Chapter 3,** we described the ethnic differences in knowledge about and actual use of folic acid supplements before or during pregnancy, and investigated in particular the role of language proficiency as a determinant of knowledge and use in these groups. Analyses were based on 8050 women for whom data on determinant and outcome variables were complete. We found that periconceptional use of folic acid was particularly low among women born in Turkey (25%), Morocco (24%), Ghana (21%), or another non-Western country (41%), as compared to women born in the Netherlands (86%) or another Western country (78%). For Surinamese and Antillean women, rates of use were 51% and 60%, respectively. In line with the "stages of change" theory, we observed that knowledge largely determined the use of supplements, both in Western and non-Western ethnic groups. Knowledge, in turn, depended on two factors: language proficiency (in the groups with a mother tongue other than Dutch) and educational level (all groups). These results imply that for the promotion of folic acid use among non-Dutch-speaking foreign-born women, easily accessible and linguistically appropriate information is required.

In **Chapter 4**, we reported on the ethnic differences in n-3 and n-6 fatty acid status in pregnancy. Fatty acid results were available for 4336 participants of the biomarker study; additional restriction to the main ethnic groups of Amsterdam provided the final sample analysis for this study of n = 3284. Compared to Dutch women, women from Surinamese, Antillean, Turkish, and Moroccan origin (including 2nd generation) had generally lower concentrations of n-3 fatty acids, but higher concentrations of n-6 fatty acids. Ghanaian women, in contrast, had higher concentrations of eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), but generally lower n-6 fatty acid concentrations. Given the interrelated metabolisms of the n-3 and n-6 fatty acids, we investigated whether differences in fish consumption (as a major source of EPA and DHA) could explain differences observed

for EPA and DHA, as well as dihomo-γ-linolenic acid (DGLA, 20:3n-6), and arachidonic acid (AA, 20:4n-6). On the group level, fish consumption did not attenuate the differences observed for DGLA and AA, and only modestly affected the comparisons for EPA and DHA. *Post hoc* analyses suggested that metabolic differences, influencing the conversion of linoleic acid and α-linolenic acid to their longer-chain derivatives, could be more relevant.

Chapter 5 addressed the issue of ethnic heterogeneity in birth weight. Dutch, Surinamese, Antillean, Turkish, Moroccan, Ghanaian, and other non-Dutch women were compared with respect to the term birth weight distribution of their singleton liveborn infants (n = 7118); the ethnic differences were subsequently investigated in terms of the underlying constitutional and environmental factors. For all ethnic minority groups (1st and 2nd generations) the crude birth weight distributions (standardized for gestational age only) were shifted to lower birth weights (−21 g to −255 g) compared to the Dutch distribution. Adjustment for constitutional factors (infant sex, maternal parity, age, and height) substantially decreased these ethnic disparities, whereas adjustment for environmental factors (education, cohabitant status, maternal BMI, smoking, alcohol consumption, depression, and work stress) provided little additional explanation. Infants of Surinamese, Antillean and Ghanaian women remained, after full adjustment, significantly smaller than the Dutch newborns (−98 g to −159 g). The prognostic significance as well as the underlying mechanisms of these disparities require further research. Explanations for these persisting disparities may include nutrition, but other pathways need to be explored as well.

Finally, Chapters 6 and 7 reported on the role of maternal nutrition in fetal growth. In **Chapter 6**, we evaluated the so-called folate depletion hypothesis, which claims folate depletion to be the main contributor to the excess risk of fetal growth restriction at short interpregnancy intervals. For this purpose, we examined whether folic acid supplement use modified the association of short interpregnancy intervals with term birth weight and SGA risk. First-time deliveries were excluded and analyses were restricted to singleton liveborn births (n = 3153). Among nonusers of folic acid supplements, each unit increase in interpregnancy interval on a natural log (ln) scale (range 1–24 months) was associated with a 165 g increase in birth weight and an approximately 60% decrease in SGA risk, after adjustment for relevant covariables. This corresponded to a birth weight difference of at least −230 g, and an odds ratio for SGA of at least 3.9, for infants born to women with intervals of 6 months or shorter compared with infants born to women with intervals of 2 years. In contrast, no significant interval-associated decrease in birth weight or increase in SGA risk was observed among supplement users. Although our results corroborate the folate depletion hypothesis, prospective postnatal interventions studies are required to provide decisive evidence.

In **Chapter 7,** we explored the potential growth effects of the maternal fatty acid profile in early pregnancy, by examining the association of maternal n-3 and n-6 fatty acid

concentrations with infant birth weight and SGA risk at term (singleton liveborn infants, n = 3704). In addition, this study investigated the role of elaidic acid (ELA, 18:1n-9*trans*), the main industrial *trans* fatty acid in the diet. In univariate analysis, all individual fatty acids were significantly associated with birth weight and SGA risk, but in a nonlinear manner. For all n-3 fatty acids and DGLA (20:3n-6), birth weight was decreased (and SGA risk increased) at low concentrations, for the other n-6 fatty acids and elaidic acid, in contrast, birth weight was decreased at high concentrations. After adjustment for physiologic, lifestyle-related and sociodemographic factors, low concentrations of most n-3 fatty acids and DGLA, and high concentrations of AA remained associated with lower birth weight (−52 g to −57 g) and/or SGA risk (odds ratios 1.38 to 1.50). Infants of the 7% women with the most adverse fatty acid profile, as based on the combination of maternal n-3, n-6 and *trans* FA concentrations, were on average 125 g lighter and twice as likely to be SGA. Given these results, a study investigating the feasibility as well as the potential benefits of a dietary intervention seems worthwhile.

In **Chapter 8**, we reflected on the main finding of our studies. The potential relevance of ethnic differences in nutrition to ethnic disparities in birth weight was explored and the lessons learned for future research were summarized. In addition, we considered the implications of our results to public health policy and perinatal care, and discussed some methodological issues. From our results, three general conclusions can be drawn:

(1) Whereas nutritional factors were shown to be relevant to fetal growth, their role in explaining ethnic disparities in birth weight appears to be modest. Although preventing inadequate maternal nutrition is important at an individual level, caution is advised with regard to expectations of intervention effects at the population level. With respect to the n-3 and n-6 essential polyunsaturated fatty acids, interventions aimed at improving the maternal profile of these fatty acids still require further study, which should take into account the dynamics of fatty acid metabolism. Improving the maternal periconceptional folate status, still of undisputed importance for preventing neural tube defects, may, in a multi-ethnic society, best be achieved by fortification, but this option is still being debated in the Netherlands. Promising alternative strategies are the employment of ethnic health advisors in maternal and child health care centers and language courses for immigrants: both measures allow for the dissemination of linguistically appropriate, comprehensive information about folic acid use as well as family planning, the latter being an important measure to prevent folate depletion in a subsequent pregnancy.

(2) When examining ethnic disparities in health and disease, three concepts of ethnicity are relevant: (a) race/genetic constitution; (b) sociocultural orientation; and (c) migrant status. Which concepts to consider depends on their relative importance to the health problem and research question at stake. For example, research into the role of nutrition in explaining ethnic disparities in perinatal health requires an understanding of the dietary (sociocultural) as well as metabolic (genetic) aspects of nutrition. The same is true when deciding on a public health

strategy. Some problems (e.g., lower birth weight) may require a more tailored intervention restricted to specific subgroups (women of African descent), whereas other problems (folic acid supplement use) may benefit best from a universal measure (language education for all immigrants).

(3) It can be assumed that the observed ethnic disparities, both in birth weight and the determinants thereof, are also relevant to ethnic disparities in later life. In time, the ABCD study will provide more insight into the consequences of an adverse fetal environment to health and disease in childhood, and into the extent to which ethnic health differences are indeed explained by ethnic differences in the fetal environment. As such, the study will form a basis for developing and implementing early life interventions.

Samenvatting

Geboortegewicht is één van de belangrijkste uitkomstmaten van een zwangerschap, en niet alleen omdat het iets zegt over de gezondheid van moeder en kind tijdens de zwangerschap. Geboortegewicht lijkt ook een belangrijke indicator voor de latere gezondheid van kinderen te zijn: hoe lager het geboortegewicht, hoe groter het risico op ziekten en aandoeningen op latere leeftijd (de zogeheten *fetal origins of disease* hypothese). Wereldwijd bestaan er grote verschillen in de geboortegewichtverdelingen tussen verschillende etnische groepen; binnen één land (bijv. Nederland) zien we vaak dat een laag geboortegewicht vooral voorkomt bij kinderen die behoren tot een zogenaamde etnische minderheid (bijv. Surinamers of Marokkanen). Vanuit het oogpunt van preventie is het belangrijk om meer inzicht te krijgen in de factoren die de foetale groei (de groei van het ongeboren kind) beïnvloeden, vooral als deze invloed negatief is, en verbonden is met slechtere uitkomsten. Op basis van dat inzicht kunnen dan maatregelen worden genomen om verschillen in geboortegewicht tussen etnische groepen te verkleinen. Opvallend genoeg gaan etnische verschillen in geboortegewicht vaak samen met etnische verschillen in maternale voeding (d.w.z. de voedingsinname of voedingsstatus van de moeder), wat de vraag oproept in hoeverre de maternale voeding een verklarende factor is voor verschillen in geboortegewicht naar etniciteit.

Het doel van dit proefschrift, zoals beschreven in **hoofdstuk 1**, was om meer inzicht te krijgen in de rol van maternale voeding als verklaring voor etnische verschillen in foetale groei. Daarbij werden, als maat voor foetale groei, de volgende uitkomstmaten meegenomen: het geboortegewicht bij een à terme geboorte (d.w.z. vanaf 37 weken zwangerschap) en het vóórkomen van *small-for-gestational-age (SGA)* geboortes onder de à terme geboortes (d.w.z. geboortes waarbij het geboortegewicht valt binnen de laagste 10 procent van de geboortegewichtverdeling, rekening houdend met het geslacht van het kind, de zwangerschapsduur, en het aantal eerdere zwangerschappen van de moeder). Onderzocht zijn: (1) de samenhang tussen etniciteit en maternale voeding (en determinanten daarvan); (2) de samenhang tussen etniciteit en geboortegewicht (en verklarende factoren daarin, anders dan voeding); en (3) de rol van maternale voeding als determinant van geboortegewicht. Onze aandacht ging daarbij uit naar twee specifieke voedingsfactoren: ten eerste foliumzuur en ten tweede de n-3 en n-6 essentiële vetzuren linolzuur en α-linoleenzuur en hun derivaten, de lange-keten meervoudig onverzadigde vetzuren (*long-chain polyunsaturated fatty acids, LC-PUFAs)*.

De studies beschreven in dit proefschrift waren onderdeel van de Amsterdam Born Children and their Development (ABCD) studie, een grootschalig prospectief onderzoek dat wordt uitgevoerd in Amsterdam. Van januari 2003 tot maart 2004 werden 12.373 zwangere vrouwen in Amsterdam tijdens hun eerste bezoek aan de gynaecoloog, verloskundige, of huisarts benaderd om mee te doen aan de ABCD-studie. Van deze vrouwen besloot

67% deel te nemen (d.w.z. 8.266 vrouwen). Al deze vrouwen vulden een uitgebreide vragenlijst in over o.a. voeding (gebruik van foliumzuursupplementen en visconsumptie), sociaaldemografische factoren (zoals leeftijd en opleidingsniveau), medische achtergrond, eerdere zwangerschappen, leefstijl en psychosociale factoren. In de studiepopulatie waren de belangrijkste etnische groepen in Amsterdam vertegenwoordigd, namelijk de Nederlandse, Surinaamse, Antilliaanse (inclusief Arubaanse), Turkse, Marokkaanse en Ghanese vrouwen (gedefinieerd aan de hand van geboorteland). Van de deelneemsters deed 53% ook mee aan de zogeheten ABCD *biomarker* studie, waarbij de voedingsstatus van de moeder werd gemeten in het bloed. Daarvoor stonden vrouwen een extra bloedmonster af tijdens de bloedafname die routinematig plaatsvindt bij de eerste zwangerschapscontrole, waarna de bloedmonsters naar het Streeklaboratorium van Amsterdam werden gestuurd voor verwerking en verdere opslag tot analyse.

Omdat de validiteit van de bloedbepalingen afhangt van de methode van bloedafname en verwerking (wel/geen directe verwerking van het afgenomen bloed), onderzocht onze eerste studie de validiteit van onze pragmatische methode (**hoofdstuk 2**). Van 41 vrouwelijke vrijwilligers werden bloedmonsters afgenomen (20 vrouwen voor serummonsters, 21 vrouwen voor plasmamonsters), die vervolgens, in het donker, werden opgeslagen bij kamertemperatuur. Eén monster werd binnen 2 uur na afname verwerkt (baseline), de overige monsters werden 2, 4, 24, 26, 28 en 96 uur later verwerkt. Uit de studie bleek dat na 96 uur de concentraties van CRP, ferritine, retinol, alle n-3 vetzuren en de meeste n-6 vetzuren minder dan 10% waren veranderd. Na 96 uur waren de concentraties van foliumzuur en linolzuur significant afgenomen (respectievelijk met 16,5% en 14,0%), terwijl de concentratie van adrinezuur (22:4n-6) significant was toegenomen (16,2%). De tijdgerelateerde veranderingen waren echter nooit relevant voor de 28-uurs betrouwbaarheid of validiteit (28 uur was de maximale toegestane tijd tussen afname en verwerking in de ABCD-studie): intraclass correlatiecoëfficiënten waren allemaal 0,9 of hoger en Spearman rank correlatiecoëfficiënten (berekend volgens de zogeheten *bootstrap* methode) 0,8 of hoger. Dat betekent dat, zolang de tijd tussen afname en verwerking 28 uur of minder is, dergelijke bloedbepalingen valide gebruikt kunnen worden in standaard epidemiologische analyses.

De hoofdstukken 3 en 4 waren vervolgens gericht op de etnische verschillen in voeding. In **hoofdstuk 3** beschreven we de etnische verschillen in kennis over en gebruik van foliumzuursupplementen vóór en tijdens de zwangerschap. Daarbij onderzochten we vooral in hoeverre de taalvaardigheid van invloed was op zowel kennis als gebruik. De statistische analyses waren gebaseerd op data van 8.050 vrouwen, voor wie we alle informatie hadden die nodig was voor het onderzoek (zoals het supplementgebruik en de taalvaardigheid). Uit het onderzoek bleek dat gebruik van foliumzuursupplementen rondom de conceptie vooral laag was onder vrouwen geboren in Turkije (25%), Marokko (24%), Ghana (21%), of een ander

niet-Westers land (41%), vergeleken met vrouwen geboren in Nederland (86%), of een ander Westers land (78%). Van de Surinaamse en Antilliaanse vrouwen gebruikte respectievelijk 51% en 60% foliumzuur. In overeenkomst met het theoretische *stages of change* model, zagen we dat (een gebrek aan) kennis in grote mate het (lage) gebruik van foliumzuur bepaalde, zowel in de groep Westerse als in de groep niet-Westerse vrouwen. De kennis over foliumzuur werd bepaald door (1) de taalvaardigheid (voor vrouwen met een moedertaal anders dan Nederlands) en (2) het opleidingsniveau (voor alle vrouwen). Om het gebruik van foliumzuur onder niet-Nederlands sprekende vrouwen te bevorderen, zijn er daarom maatregelen nodig die niet alleen makkelijk toegankelijk zijn (een eenvoudige en duidelijke boodschap), maar ook rekening houden met de taalvaardigheid van deze vrouwen.

In **hoofdstuk 4** rapporteerden we de verschillen in vetzuurstatus naar etniciteit. Van 4.336 deelneemsters van de ABCD-studie waren de n-3 en n-6 vetzuurconcentraties gemeten, maar uiteindelijk zijn de gegevens van 3.284 vrouwen meegenomen in het onderzoek (alleen die deelneemsters die behoorden tot één van de zes grootste etnische groepen). Over het algemeen hadden vrouwen van Surinaamse, Antilliaanse, Turkse en Marokkaanse afkomst (1^{e} en 2^{e} generatie samen), in vergelijking met Nederlandse vrouwen, lagere concentraties van de n-3 vetzuren, en hogere concentraties van de n-6 vetzuren. Ghanese vrouwen hadden daarentegen hogere concentraties van de n-3 vetzuren eicosapentaeenzuur (EPA, 20:5n-3) en docosahexaeenzuur (DHA, 22:6n-3) in het bloed, maar lagere concentraties van de n-6 vetzuren. Omdat het metabolisme van de n-3 vetzuren sterk gerelateerd is aan dat van de n-6 vetzuren (en vice versa), hebben we ook onderzocht in hoeverre visconsumptie (een belangrijke bron van EPA en DHA) verschillen zou kunnen verklaren in zowel EPA en DHA, als in di-homo-γ-linoleenzuur (DGLA, 20:3n-6) en arachidonzuur (AA, 20:4n-6). Visconsumptie bleek op groepsniveau wel een deel te verklaren van de verschillen in EPA en DHA concentraties, maar bijna niets van de verschillen in DGLA en AA concentraties. De resultaten van *post hoc* analyses (analyses die achteraf, na het beantwoorden van de originele onderzoeksvraag worden uitgevoerd) gaven aan dat wellicht metabole verschillen tussen etnische groepen (verschillen in de omzetting van linolzuur en α-linoleenzuur naar hun derivaten) belangrijker zijn dan inname verschillen.

Hoofdstuk 5 betrof de etnische verschillen in geboortegewicht. In dit onderzoek werden de geboortegewichten van de kinderen (levendgeboren, vanaf 37 weken zwangerschap) van 7.118 moeders van Nederlandse, Surinaamse, Antilliaanse, Turkse, Marokkaanse, Ghanese en overige niet-Nederlandse afkomst vergeleken. Vervolgens werd getracht de etnische verschillen te verklaren door te kijken naar de onderliggende determinanten (de zogenoemde constitutionele factoren en omgevingsfactoren). Voor zowel 1^{e} als 2^{e} generatie allochtone vrouwen gold dat het geboortegewicht van hun kinderen (gestandaardiseerd voor zwangerschapsduur) lager was dan dat van Nederlandse kinderen (gemiddeld 21 gram tot 255 gram). Na correctie voor constitutionele factoren (geslacht van het kind, en

leeftijd, lengte en aantal eerdere zwangerschappen van de moeder) werden de verschillen substantieel kleiner. Correctie voor omgevingsfactoren [opleidingsniveau, huishoudsituatie (alleenwonend of samenwonend), BMI, rookgedrag, alcoholconsumptie, depressiviteit en werkgerelateerde stress] daarentegen verklaarde weinig van de etnische verschillen. Na volledige correctie (voor zowel de constitutionele als de omgevingsfactoren) waren kinderen van Surinaamse, Antilliaanse en Ghanese vrouwen significant kleiner dan Nederlandse kinderen (geboortegewicht 98 gram tot 159 gram lager). Verder onderzoek is nodig, niet alleen om de relevantie van deze verschillen voor latere gezondheid te bepalen, maar ook om het onderliggende mechanisme van dit resterende verschil te ontrafelen. Voeding speelt mogelijk een rol, maar ook andere verklaringen moeten onderzocht worden.

Tot slot betroffen de hoofdstukken 6 en 7 de rol van maternale voeding in foetale groei. In **hoofdstuk 6** evalueerden we de zogenoemde foliumzuurdepletie hypothese, die claimt dat een tekort aan foliumzuur in het lichaam van de moeder in belangrijke mate de oorzaak is van een hoger risico op intra-uteriene groeivertraging bij korte zwangerschapsintervallen (d.w.z. een korte tijdsduur tussen de geboorte van een kind en de conceptie van een volgend kind). Om dit te onderzoeken, hebben we gekeken naar het gebruik van foliumzuursupplementen en in hoeverre dat van invloed was op het verband tussen korte zwangerschapsintervallen en geboortegewicht en SGA. Daarbij werden vrouwen die zwanger waren van hun eerste kind geëxcludeerd; de analyses betroffen uiteindelijk 3.153 à terme levendgeboren kinderen en hun moeders. Uit de studie bleek dat onder vrouwen die geen foliumzuursupplementen gebruikten het zwangerschapsinterval sterk gerelateerd was aan het geboortegewicht en het risico op een SGA kind: als het zwangerschapinterval met 1 eenheid toenam (logaritmische schaal), nam het geboortegewicht met 165 gram toe, en het SGA risico met ongeveer 60% af (na correctie voor andere factoren). Dat betekent, omgerekend naar een tijdseenheid in maanden, dat onder de niet-supplementgebruiksters de pasgeborenen van vrouwen met een zwangerschapsinterval van 6 maanden of korter ten minste 230 gram lichter waren dan de pasgeborenen van vrouwen met een zwangerschapsinterval van 2 jaar, en dat hun risico om een SGA kind te zijn ten minste 3,9 keer zo groot was. Een dergelijke samenhang werd daarentegen niet gevonden onder vrouwen die wel foliumzuursupplementen slikten. Onze resultaten ondersteunen daarmee de depletie hypothese, al zijn voor een definitieve bevestiging idealiter experimentele suppletiestudies nodig.

In **hoofdstuk 7** onderzochten we het verband tussen de maternale vetzuurstatus vroeg in de zwangerschap en de foetale groei. Dat deden we door de samenhang tussen de maternale n-3 en n-6 vetzuurconcentraties en het geboortegewicht dan wel risico op SGA te analyseren voor 3.704 à terme, levengeboren kinderen en hun moeders. Daarbij onderzochten we ook de rol van elaïdinezuur (ELA, 18:1n-9 *trans*), het belangrijkste *trans* vetzuur (en daarmee een van de ongezondste vetzuren) in onze voeding. Uit de univariate analyse (d.w.z. niet gecorrigeerd voor andere factoren die geboortegewicht beïnvloeden) bleek dat alle vetzuren

samenhingen met geboortegewicht en SGA, maar dat die samenhang niet lineair was. Voor de n-3 vetzuren en het n-6 vetzuur DGLA gold, dat het geboortegewicht lager (en SGA risico hoger) was als concentraties laag waren, voor de overigen n-6 vetzuren en ELA gold echter dat geboortegewicht lager (en SGA risico hoger) was als concentraties hoog waren. Na correctie voor fysiologische, leefstijlgerelateerde, en sociaaldemografische factoren, bleven lage concentraties van de meeste n-3 vetzuren en het n-6 vetzuur DGLA, alsmede hoge concentraties van het n-6 vetzuur AA geassocieerd met een laag geboortegewicht (−52 gram tot −57 gram) en/of SGA [odds ratio (een maat voor het risico): 1,38 tot 1,50]. De pasgeborenen van de 7% vrouwen die het meest ongunstige vetzuurprofiel hadden (gebaseerd op de combinatie van de n-3, n-6 en *trans* vetzuur concentraties), waren gemiddeld 125 gram lichter en twee keer vaker SGA dan pasgeborenen van vrouwen met het meest gunstige profiel. Deze resultaten geven aan dat het de moeite waard is om meer onderzoek te doen naar de preventie van een laag geboortegewicht door aanpassing van de maternale vetzuurstatus.

Hoofdstuk 8 presenteerde de reflectie op onze bevindingen. In deze algemene discussie exploreerden we de mogelijke relevantie van etnische verschillen in voeding voor etnische verschillen in geboortegewicht en bespraken we de leerpunten die uit ons onderzoek naar voren kwamen. Daarnaast bespraken we de implicaties van onze resultaten voor het volksgezondheidsbeleid en de perinatale zorg, en tot slot bediscussieerden we de methodologische beperkingen. Ons resultaten leidden tot de volgende drie conclusies:
(1) Hoewel voedingsfactoren van belang zijn voor foetale groei, lijkt hun rol in de verklaring van etnische verschillen beperkt te zijn. Op individueel niveau blijft preventie van een ongezonde voeding uiteraard van belang, maar van effecten van een dergelijke preventie op groepsniveau moeten we niet te veel verwachten. Wat betreft de n-3 en n-6 vetzuren is nog nader onderzoek nodig naar de geboortegewicht effecten van interventies die de maternale vetzuurstatus verbeteren. Daarbij moet vooral rekening worden gehouden met het vetzuurmetabolisme (immers het verbeteren van de concentratie van één vetzuur heeft invloed op de concentraties van de andere vetzuren). Het verbeteren van de maternale foliumzuurstatus, dat hoe dan ook van belang is voor de preventie van neuraalbuisdefecten (bijv. het zogeheten open ruggetje), lijkt in een multiculturele samenleving nog het best haalbaar via fortificatie (het toevoegen van foliumzuur aan voedingsmiddelen). Fortificatie is echter nog steeds een discussiepunt in Nederland. Veelbelovende alternatieven zijn de inzet van voorlichters eigen taal en cultuur in consultatiebureaus of Ouder-en-Kindcentra, en het aanbieden van taalcursussen voor immigranten: via beide maatregelen kan een eenvoudige boodschap, desgewenst in de eigen taal, worden uitgedragen naar de vrouwen toe, niet alleen met betrekking tot foliumzuur maar ook met betrekking tot gezinsplanning. Met name het laatste is een voorwaarde om, in een volgende zwangerschap, een tekort aan foliumzuur te kunnen voorkomen.
(2) In onderzoek naar etnische verschillen in gezondheid of ziekte, zijn drie concepten van etniciteit relevant: (a) ras/genetische constitutie; (b) sociaalculturele oriëntatie; en (c)

migratiestatus. Met welke concepten rekening moet worden gehouden, hangt af van de specifieke onderzoeksvraag en de mate waarin ze bijdragen aan het gezondheidsprobleem. Onderzoek naar de rol van voeding in de verklaring van etnische verschillen in perinatale gezondheid, bijvoorbeeld, vraagt om inzicht in zowel de sociaalculturele aspecten (het eetpatroon) als de genetische aspecten (het metabolisme). Hetzelfde geldt met betrekking tot het ontwikkelen van een interventie binnen het volksgezondheidsbeleid. Sommige problemen (bijvoorbeeld laag geboortegewicht) vragen om een aanpak gericht op een specifiek doelgroep (vrouwen van Afrikaanse afkomst), terwijl andere problemen (het gebruik van foliumzuursupplementen) meer profiteren van een universele benadering (taalcursussen voor alle immigranten).

(3) Tot slot is het waarschijnlijk dat de geobserveerde etnische verschillen, zowel in geboortegewicht als in determinanten daarvan, relevant zijn voor verschillen in latere gezondheid. In de nabije toekomst zal de ABCD-studie meer inzicht geven in de gevolgen van een ongunstige foetale ontwikkeling voor gezondheid en ziekte op latere (kinder-) leeftijd, en in de mate waarin etnische verschillen in latere gezondheid verklaard kunnen worden door etnische verschillen tijdens de zwangerschap. Daarmee vormt de ABCD-studie een basis voor de ontwikkeling en implementatie van vroege interventies.

Abbreviations
Contributors

Abbreviations

AA	Arachidonic acid
ABCD	Amsterdam Born Children and their Development
ALSPAC	Avon Longitudinal Study of Parents and Children
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
B	Beta (unstandardized regression coefficient)
BMI	Body mass index
CES-D	Center for Epidemiologic Studies depression scale
CI	Confidence interval
CRP	C-reactive protein
DGLA	Dihomo-γ-linolenic acid
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
DPA	Docosapentaenoic acid
EDTA (K2)	Ethylene diamine tetracetic acid dipotassium
EPA	Eicosapentaenoic acid
FA	Fatty acid
FFQ	Food frequency questionnaire
GA	Gestational age
ICC	Intraclass correlation coefficient
JCQ	Job content questionnaire
LBW	Low birth weight
LC-PUFA	Long-chain polyunsaturated fatty acid
Ln	Natural logarithm
OR	Odds ratio
PRN	The Netherlands perinatal registry
Q	Quintile
SD	Standard deviation
SE	Standard error
SGA	Small for gestational age
SPSS	Statistical Package for the Social Sciences

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About the author

Manon van Eijsden was born very appropriate for gestational age (3580 grams at 40 weeks' gestation) on the 2nd of September 1978 in Rotterdam, the Netherlands. She grew up in Brielle, a small town nearby her place of birth, where she graduated from high school (van Maerlant College) in 1996. In the same year, she started with the MSc program of Nutrition and Health at Wageningen University in Wageningen, the Netherlands. From September 1999 until September 2000, she interrupted her study to become one of the six members of the "Commissie Algemene Introductie Dagen", a committee that organizes the introduction program for first year students at the university. In 2001, after writing her MSc thesis at the department of Human Nutrition ("The effect of vitamin E and multivitamin/-mineral supplementation on the incidence of acute infectious diseases in an elderly population") she went abroad to work as a Survey and Advocacy Associate at the School Feeding Support Unit of the World Food Programme (United Nations) in Rome, Italy. Her activities there were diverse: she conducted the so-called School Feeding Survey in Mozambique, the Gambia, Surinam, and the Netherlands Antilles, was involved in a pilot study to evaluate school feeding programs in Uganda, and coordinated two international workshops for WFP colleagues about the latter in Uganda and Nepal. Back in the Netherlands (at the end of 2001), she returned to the field of epidemiologic research and started another internship at the department of Epidemiology of the Municipal Health Service of Utrecht, where she investigated and reported on the health situation of the youth in Utrecht. In June 2002, she graduated her MSc study with honors and continued working in Utrecht as a junior epidemiologist. A few months later, in January 2003, she changed her workplace in Utrecht for the Public Health Service of Amsterdam, where she joined the Amsterdam Born Children and their Development (ABCD) study group for a PhD project. Her research, described in this thesis, was conducted at the department of Epidemiology, Documentation, and Health Promotion of the Public Health Service of Amsterdam and the department of Social Medicine of the Academic Medical Center (University of Amsterdam). During her PhD project, Manon presented results of the ABCD study at various international congresses, and in November 2007, she received an Early Career Researcher Award at the 5th World Congress on Developmental Origins of Health and Disease for her abstract on the association between interpregnancy interval, folate depletion, and birth weight. Since January 2008 she has a permanent position as an epidemiologist at the Public Health Service. In this position, she will continue her work in the ABCD project, as a researcher as well as coordinator of the next phases of this long-term birth cohort study.

Over de auteur

Met het mooie geboortegewicht van 3580 gram (bij 40 weken zwangerschapsduur), werd Manon van Eijsden op 2 september 1978 in Rotterdam geboren. Ze groeide op in een stadje vlakbij haar geboorteplaats, Brielle, alwaar ze in 1996 haar VWO diploma behaalde aan het Van Maerlant College. In datzelfde jaar begon ze met haar studie Voeding en Gezondheid aan destijds de Landbouwuniversiteit (nu Wageningen Universiteit) in Wageningen. In het studiejaar 1999/2000 onderbrak ze haar studie om lid te worden van de Commissie Algemene Introductie Dagen, een groep van zes studenten die de introductiedagen voor de nieuwe eerstejaarsstudenten organiseerde. In 2001, na het afronden van een epidemiologisch afstudeervak bij de vakgroep Humane Voeding ("The effect of vitamin E and multivitamin/-mineral supplementation on the incidence of acute infectious diseases in an elderly population"), vertrok ze voor 9 maanden naar het buitenland om als "Survey and Advocacy Associate" aan de slag te gaan bij het World Food Program (WFP) van de Verenigde Naties in Rome, Italië. In die hoedanigheid voerde ze een survey uit naar schoolvoeding in onder andere Mozambique, Gambia, de Nederlandse Antillen en Suriname, was ze betrokken bij een pilotstudie in Uganda ter evaluatie van schoolvoedingsprogramma's, en coördineerde ze een tweetal workshops daarover in Uganda en Nepal. Eind 2001 keerde ze terug naar Nederland en terug naar de epidemiologie: haar derde en laatste afstudeerproject, bij de GG&GD in Utrecht, betrof een onderzoek naar de gezondheid van de Utrechtse jeugd ("De Utrechtse jeugd gezond? Een onderzoek naar de gezondheid van de Utrechtse jeugd: trends en sociaaldemografische verschillen"). In juni 2002 ontving ze *cum laude* haar ingenieurstitel en werd ze aangesteld als junior onderzoeker bij de GG&GD in Utrecht. Enkele maanden later verruilde ze het Utrechtse werkveld voor Amsterdam, en ging ze aan de slag als promovenda bij de Amsterdam Born Children and their Development (ABCD) studie. Haar promotieonderzoek, beschreven in dit proefschrift, voerde ze uit binnen het cluster Epidemiologie, Documentatie en Gezondheidsbevordering van de GGD Amsterdam en de afdeling Sociale Geneeskunde van het Academisch Medisch Centrum van de Universiteit van Amsterdam. Tijdens haar promotietraject heeft Manon op verschillende internationale congressen resultaten van de ABCD-studie gepresenteerd. Eind 2007 ontving ze tijdens het 5e World Congress on Developmental Origins of Health and Disease een Early Career Researcher Award voor haar bijdrage over de relatie tussen zwangerschapsinterval, foliumzuurdepletie en geboortegewicht. Sinds januari 2008 heeft ze een vaste aanstelling bij de GGD Amsterdam. Na haar promotie zal ze haar werk bij de ABCD-studie dan ook voortzetten, niet alleen als onderzoeker maar ook als coördinator van de volgende onderzoeksfases van dit langlopende geboortecohort.

List of publications

van Eijsden M, Hornstra G, van der Wal MF, Bonsel GJ. Ethnic differences in early pregnancy maternal n-3 and n-6 fatty acid concentrations not explained by fish consumption (submitted).

van Eijsden M, Smits LJ, van der Wal MF, Bonsel GJ. Association between short interpregnancy intervals and term birth weight: the role of folate depletion. Am J Clin Nutr (in press).

van Eijsden M, Hornstra G, van der Wal MF, Vrijkotte TG, 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.

van Eijsden M, van der Wal MF, Bonsel GJ. Folic acid knowledge and use in a multi-ethnic pregnancy cohort: the role of language proficiency. BJOG 2006;113:1446–51.

van Eijsden M, van der Wal MF, Hornstra G, Bonsel GJ. Can whole-blood samples be stored over 24 hours without compromising stability of C-reactive protein, retinol, ferritin, folic acid and fatty acids in epidemiologic research? Clin Chem 2005;51:230–2 (erratum in Clin Chem 2007;53:2226).

van Eijsden M, de Geus G, van Ameijden EJ. De gezondheid van de Utrechtse jeugd: trends en sociaaldemografische verschillen (The health situation of the youth in Utrecht: temporal trends and socio-demographic differences). TSG tijdschrift voor gezondheidswetenschappen 2004;82:12–20 (in Dutch).

Berkenpas ME, van Eijsden M, van der Wal MF. Borstvoeding in een multi-etnische populatie: de rol van de aanstaande vader en grootmoeder (Breastfeeding in a multi-ethnic population: the role of the future dad and grandmother) (in revision; in Dutch).

Goedhart G, van Eijsden M, van der Wal MF, Bonsel GJ. Ethnic differences in term birth weight; the role of constitutional and environmental factors. Paediatr Perinatal Epidemiol (in press).

Goedhart G, van Eijsden M, van der Wal MF, Bonsel GJ. Ethnic differences in preterm birth and its subtypes: the effect of a cumulative risk profile. BJOG 2008;115:710–9.

Goedhart G, van Eijsden M, van der Wal MF, Vrijkotte TG, Bonsel GJ. Prematuriteit en laag geboortegewicht: wat zijn de risicofactoren? (Prematurity and low birth weight: what are the risk factors?). Vroeg 2007;24(1):6–9 (in Dutch).

Hornstra G, van Eijsden M, Dirix C, Bonsel GJ. Trans fatty acids and birth outcome: some first results of the MEFAB and ABCD cohorts. Atheroscler Suppl 2006;7(2):21–3.

Jansen E, van Eijsden M, van der Wal M, Bonsel G. Foliumzuurinname van zwangeren nog te laag (Folic acid supplement use among pregnant women remains low). Voeding Nu 2006;8(9):18–20 (in Dutch).

Tromp M, van Eijsden M, Ravelli AC, Bonsel GJ. Anonymous non-response analysis in the ABCD-cohort study enabled by probabilistic record linkage (submitted).

Vrijkotte TG, van Eijsden M, van der Wal MF, Bonsel GJ. First trimester employment, working conditions and birth weight: a prospective cohort study (submitted).

van der Wal MF, van Eijsden M, Bonsel GJ. Stress and emotional problems during pregnancy and excessive infant crying. J Dev Behav Pediatr 2007;28:431–7.

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Dankwoord

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