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 US5-10 and the Asian populations in the UK11-13 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) oft en consume lower-quality diets than women of European/Caucasian origin,20-25 which raises the question: are diff erences in maternal nutrition the key to explaining ethnicity-related diff erences 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{31,32} Current knowledge pinpoints the fetal growth trajectory as being set very early in gestation;\textsuperscript{33,34} the time frame from mid- to late gestation may thus be too late for observing nutrient effects, either detrimental or beneficial.\textsuperscript{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. 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, 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. 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.

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) in mothers of growth-restricted infants, supplementation studies have yielded mixed results. Nevertheless, a preventive effect of higher folate intakes has been shown particularly in women at high risk of folate deficiency. 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. 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%. 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). 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. 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. 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 and from epidemiologic observations of longer gestations and higher birth weights in areas of high fish intake, such as the Faroe Islands. 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.

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 and maternal n-6 fatty acids may interfere with the incorporation of n-3 fatty acids in plasma phospholipids. 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.

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) 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, seemingly 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.)\textsuperscript{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.

\textbf{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


