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# Intrauterine nutritional programming of adult disease

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# Abstract

According to the intrauterine programming hypothesis, the risk of acquiring diseases in adult life is determined in part by environmental factors during embryo-fetal development. Especially maternal nutrition has been related to the risk of cancer, cardiovascular disease, diabetes and infectious diseases in the offspring in later life. In reviews on scientific evidence for the existence of intrauterine programming, nutrition in pregnancy was reported to influence the morphology of conceptual organs and therefore would modulate their physiological functioning; this is reflected in hormone homeostasis and metabolic functions. Evidence is accumulating that intrauterine programming permanently modulates the risk of acquiring diseases in adulthood. More than general dietary parameters such as caloric intake and macronutrient supply, the balance between individual nutritional components seems to play an important role. Further research into intrauterine programming should aim ultimately at determining whether education on nutrition in pregnancy can aid in the prevention of diseases causing great public health concern in society.

# Contents

# Samenvatting

De ‘intrauterine programming’ hypothese stelt dat de kans op het krijgen van ziekten op latere leeftijd mede bepaald wordt door de omstandigheden tijdens de embryonale en foetale ontwikkeling. Met name de voeding tijdens de zwangerschap wordt in verband gebracht met de kans op het krijgen van kanker, hart- en vaatziekten, diabetes, en infectieziekten door de nakomeling op latere leeftijd. In dit rapport wordt de wetenschappelijke evidentie voor het bestaan van ‘intrauterine programming’ beschreven. De laatste twee decennia is in zowel epidemiologische als dierexperimentele studies evidentie gevonden voor het bestaan van prenatale programmering. Voeding tijdens de zwangerschap is niet alleen van invloed op het geboortegewicht, maar ook op de morfologische ontwikkeling van organen als hypofyse, lever, nieren, pancreas, en mammae. Dit heeft belangrijke consequenties voor de inregeling van fysiologische systemen zoals het koolhydraat- en vetmetabolisme, de homeostase van hormonen die verband houden met de hypofyse-hypothalamus-bijnier-as, en de homeostase van de geslachtshormonen. Als gevolg daarvan worden permanente veranderingen in spiegels van onder meer circulerende geslachtshormonen, in bloeddruk en in glucosetolerantie gevonden die geassocieerd zijn met de kans op het krijgen van bijvoorbeeld borstkanker, hart- en vaatziekten, en diabetes. Met het oog op het frequente voorkomen van deze ziekten in de bevolking kan het effect van het dieet in de zwangerschap daarop mogelijk ook aanzienlijk zijn. In algemene termen is het patroon van voedselconsumptie in Nederland redelijk in overeenstemming met de normen. Echter, balansen tussen verschillende nutriënten kunnen variëren, en daarmee risico’s opleveren voor de ongeborene. De rol van de balans tussen n-3 en n-6 vetzuren bij prenatale programmering is hiervan thans wellicht het best uitgewerkte voorbeeld. Er zijn inmiddels internationaal meerdere prospectieve humane studies gaande waarin associaties tussen prenatale voeding en volwassen ziekten nader onderzocht worden. De afstand in de tijd tussen de prenatale ontwikkeling en de periode van het optreden van ziekten op volwassen leeftijd maakt dat de resultaten van deze studies pas ver in de toekomst bekend zullen zijn. Het verdient aanbeveling om onderzoek te doen teneinde de betrokken mechanismen nader in kaart te brengen. Het uiteindelijke doel daarbij is vast te stellen of gerichte dieetvoorlichting voor zwangeren een bijdrage kan leveren aan de preventie van de grote volksziekten..

# Summary

The ‘intrauterine programming’ hypothesis states that the risk of acquiring diseases in adulthood is partly determined during development *in utero*. In this context, especially nutrition during pregnancy has been related to the susceptibility of acquiring diseases such as cancer, cardiovascular diseases, diabetes mellitus and infectious diseases in adulthood.
In this report the scientific evidence for the ‘intrauterine programming’ hypothesis is discussed. During the last two decades, epidemiological as well as experimental studies stress the existence of prenatal programming in several ways. For example, nutrition during pregnancy appears to influence birth weight, but also the morphological development
of organs such as pituitary, liver, kidneys, pancreas and mammae. In turn, this has important consequences for the development and functioning of physiological systems such as carbohydrate- and fat metabolism and homeostasis of hormones related to the hypothalamus-pituitary-adrenal axis. As a consequence, permanent changes in levels of circulating hormones, glucose metabolism and blood pressure are found, which are all associated to chronic diseases. In view of the prevalence of chronic diseases in the population, the effect of the quality of the diet during pregnancy on the risk of chronic diseases in the offspring can be considerable. The pattern of food consumption in the Netherlands is generally close to the preferable dietary intakes. However, balances between individual nutrients may vary and may pose risks to the offspring. A well-studied example is the balance between n-3 and n-6 fatty acids in prenatal programming. Large prospective human studies are currently being performed in which relationships between prenatal nutrition and adult chronic disease will be clarified. However, results will not be expected in the nearby future because of the time span between the intervention and the anticipated effect. In the meantime, experimental research will be performed to elucidate underlying mechanisms. The ultimate aim of this type of research is to contribute to more well-grounded dietary guidelines for pregnant women in view of possible prevention of major chronic diseases.

# 1. Introduction

Evidence is accumulating for a role of intrauterine nutrition in programming the susceptibility for diseases in the offspring in adulthood. Nutrition during pregnancy has been linked to diseases with a high (public) health impact such as cancer, cardiovascular disease, obesity, diabetes and infectious diseases. The fetus appears to respond to the nutritional environment *in utero* in terms of anatomic and physiologic adaptations, with the aim to be adequately prepared for the nutritional conditions that will be encountered after birth. Seckl et al.[2] suggest that these adaptations may result in different 'default settings' in adult metabolism in a way which is beneficial to survival and health under continued nutritional conditions. However, when the postnatal environment is less challenging than anticipated, these default settings may be detrimental to health and may then increase the risk for cancer, cardiovascular disease, obesity, diabetes and infectious diseases [2]. The development of default morphological, physiological and metabolic settings *in utero* in response to the prenatal nutritional environment is commonly referred to as ' intrauterine programming' [1;2]. Current variations in western dietary composition may influence prenatal physiologic programming [2]. A variety of factors influence pregnancy outcome, such as genetic background, maternal disease and medication, maternal weight, maternal nutrition and other lifestyle and socioecomomic factors. Of these, maternal nutrition is an important factor that can be manipulated. The programming hypothesis suggests that optimalisation of the maternal diet during pregnancy may reduce the number of future cases of cancer, cardiovascular disease, obesity, diabetes and infectious diseases. The incidences of these diseases in developed societies are high and still increasing, and their determinants, such as adult lifestyle and genetic background are extensively studied. The 'fetal programming hypothesis' provides alternative factors which may contribute significantly to the risk of acquiring diseases in adulthood. The potentially important public health implications associated with fetal programming warrant further study of the associations between fetal nutrition and adult disease.

This report reviews the existing evidence for the concept of fetal programming of diseases, occurring in adulthood. Research on fetal programming and cancer in adulthood (chapter 2) has mainly focussed on breast cancer, a disease with an increasing incidence in many western countries. Prominent aspects shown to play a role in this association are maternal estrogen levels and the dietary fatty acid composition of the maternal diet. These mechanisms may cause permanent alterations in cellular morphology of mammary tissue, in growth factor receptor density and in signal transduction pathways, which may increase the susceptibility for breast cancer in adulthood.
Several lines of evidence suggest that maternal nutrition may have profound effects on blood pressure in later life (chapter 3). Hypertension is one of the important risk factors for cardiovascular disease. The role of growth parameters (fetal growth, birth weight, catch-up growth) and the influence of maternal diet upon birth weight is discussed. The role of maternal diet in relation to blood pressure in the offspring as well as possible underlying mechanisms (amino acids, glucocorticoids, placental characteristics and kidney development) are outlined in the second part of this chapter.
Type 2 (non-insulin-dependent) diabetes mellitus may originate through impaired development in fetal life (chapter 4). There is evidence that poor fetal growth results in a permanently reduced number of pancreatic cells and hence a reduced capacity to produce insulin. In addition, there is evidence that poor fetal growth results in insulin resistance.
In the context of other major diseases, the relationship between prenatal nutrition and susceptibility to infectious disease in the offspring is relatively unexplored (chapter 5). A human study in Gambia showed a higher mortality to infectious disease in people over 15 years old who were born in the annual hungry period. In laboratory animals, impaired immune functions in the offspring have been observed when maternal nutrition was manipulated.
The review of existing data is followed by a general discussion on the public health impact of intrauterine nutritional programming (chapter 6).

# 2. Programming of cancer

Nutrition plays a role in determining the risk of cancer. However, not only the diet during adulthood seems to contribute to the risk of cancer. Evidence is accumulating that diet during pregnancy can have effects on the susceptibility of cancer in the offspring. Estrogen levels are thought to play an important role in determing breast cancer risk. Importantly, estrogen levels *in utero* seem to be modifiable by maternal diet during pregnancy, especially with regard to dietary fatty acid composition. Since the mammary gland is largely undifferentiated before birth it may be particularly susceptible to the intrauterine estrogen levels. In this way, a 'fertile soil' for breast cancer may be created [4-6,7] The relationship between maternal diet and estrogen levels will be discussed in the first part of this chapter. In the second paragraph, mechanisms whereby estrogen may program breast cancer are discussed. Other programming mechanisms which are not related to estrogen are discussed in the last paragraph.

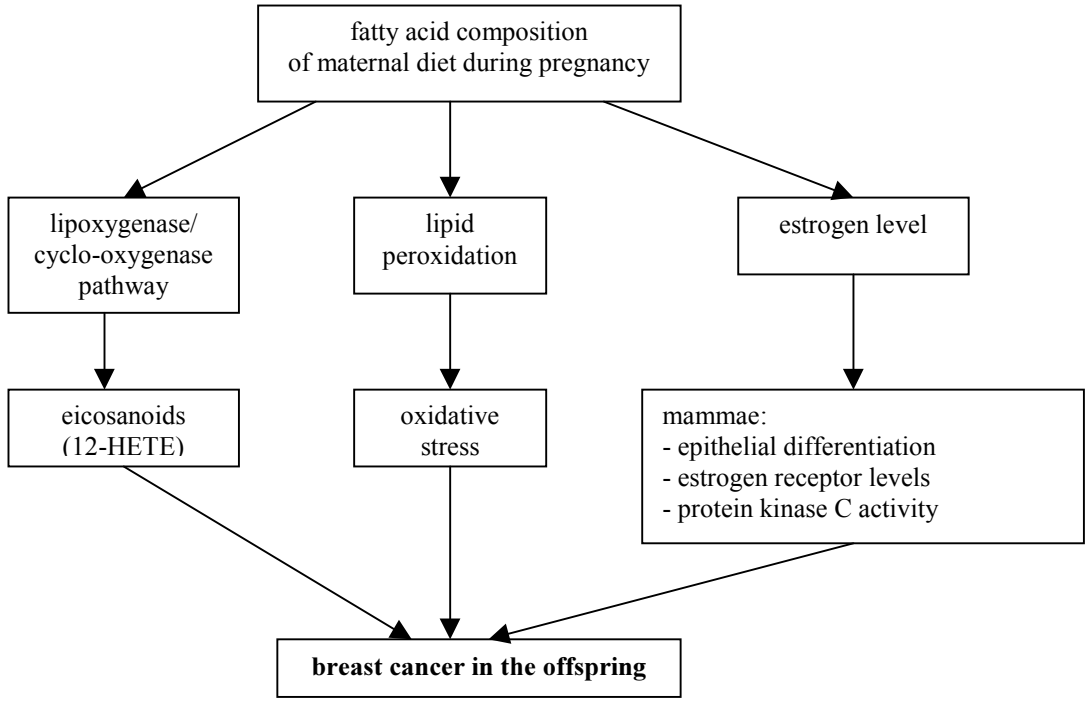


*Figure 1: Interrelationships of factors implicated in prenatal programming of breast cancer.*

## 2.1 Maternal diet and estrogen levels

Levels of circulating estrogens during pregnancy may vary up to six-fold between any two women carrying a normal pregnancy. One of the sources of these interindividual variations in estrogen level during pregnancy may be diet. Consumption of a high-fat diet in adult life has been shown to increase circulating estrogens [14;15], while estrogens are reduced by a low fat diet [16-18]. Although total fat intake has no association with breast cancer, fat composition is an important variable in terms of breast cancer risk. In a prospective study, Wolk et al. [19] found that a high intake of n-6 poly-unsaturated fatty acids (PUFAs) in adult life significantly increased breast cancer risk whereas a high intake of monounsaturated fatty acids significantly reduced breast cancer risk in a cohort of 61,471 Swedish women. The daughters of these women were not considered in this study.

Hilakivi-Clarke [20;21] investigated whether a high versus low PUFA diet during pregnancy increased the risk of mammary tumors in the female offspring in the rat. Pregnant rats were fed isocaloric diets containing 16% (low fat) or 43% (high fat) of calories from corn oil, which primarily contains the n-6 polyunsaturated fatty acid linoleic acid. They showed that a maternal diet high in n-6 PUFA increased the estrogen level as well as the incidence of DMBA-induced mammary tumors in female offspring [21]. The circulating levels of total estradiol were significantly higher (by approximately 30-100%) in the pregnant females fed an isocaloric high-fat diet, as compared to the animals fed a low-fat diet. In man, such differences in estradiol levels may be critical in terms of biological effects. For example, there is a 30% difference in the circulating estradiol levels between East Asian and Caucasian women and this may be sufficient to explain the observed differences in breast cancer risk between these populations [22].

There is experimental evidence in animals that an adult diet high in n-3 PUFA (of which α-linolenic acid is the most important fatty acid) may protect from breast cancer [24;25]. Epidemiological studies also show a decreased risk of breast cancer with increasing n-3 PUFA intake during adulthood [26]. An intervention study in humans showed, however, that a diet high in n-3 PUFA during adult life does not alter plasma estrogen levels [27]. Associations between the intake of n-3 PUFAs during pregnancy with breast cancer risk in the offspring have not been reported. Since n-3 PUFAs and n-6 PUFAs compete for the same metabolic enzyme systems, it is unclear whether the observed increased breast cancer risk in the above studies is caused by the high *in utero* levels of linoleic acid or because of a low n-3 PUFA content in the diet [28].

Although estradiol is a strong candidate for mediating the effects of maternal dietary fat on breast cancer risk, it is possible that dietary fiber may be involved. A confounding factor in some studies [20,23] using a high- and low fat diet is the difference in fiber content of the diet. Since fibers can alter estrogen metabolism, recirculation and excretion, it may have played a role in the effects noted [23].

## 2.2 Estrogen and mammary morphology

*S*everal estrogen-related mechanisms have been proposed by which the female offspring may be programmed *in utero* for breast cancer. Estrogen may have effects on the mammary gland itself or may cause changes in the gene-expression of estrogen-regulated genes.

A first effect of estrogen on the mammary gland is the down-regulation of estrogen receptor number by a high plasma estrogen level. Maternal exposure to a high-fat diet (46% of calories from fat) induced a fourfold reduction in the estrogen receptor density in the offspring's mammary gland [21;29] compared to a low fat diet (12 energy % from fat). A low mammary estrogen receptor content in the offspring was associated with a higher susceptibility to breast cancer in female mice and rats exposed to a high-fat diet *in utero* [30]. No explanation for these apparently contradictory data is given.

A second effect of estrogen in the mammary gland is an estrogen-induced increase in epithelial terminal end buds (TEBs) in the mammae anlagen in the fetus. This may increase breast cancer risk later in life [21]. In mice and rats, mammary buds are first evident in embryos at day 10-11. Rapid proliferation of the epithelial mammary structures occurs between gestation day 16 and 21. After birth, the ducts in the mammae proliferate and branch around the nipple area. These ducts end in TEBs, which are the most rapidly proliferating epithelial structures. After puberty, which begins approximately at week 5 in mice and week 6 in rats, TEBs begin to cleave into three to five smaller buds, called alveolar buds (AB) or they become atrophic and form terminal end ducts (TDs). TEBs are considered the primary targets for neoplastic transformation in the rodent mammary gland. A high exposure *in utero* to estrogen accelerates differentiation of the nipple and has been shown to induce changes in the development of TEBs resulting in an increase in their number. Interestingly, mice and

rats, exposed to estradiol during the perinatal period (days 1 to 3) exhibited a significant delay in the development and differentiation of ABs and TD; the mammae were still full of TEBs [21;31].

A third potential mechanism triggered by higher estrogen levels is a change in gene expression of the estrogen-regulated transforming growth factor α (TGF-α). An overexpression of TGF-α is generally associated with malignant transformation in the breast [32;33]. Transgenic mice overexpressing TGF-α develop abnormal mammary structures. These animals also have elevated circulating estradiol levels, suggesting that TGF-α is involved in altering normal mammary gland development [152].

## 2.3 Maternal fat intake and breast cancer: mechanisms

Besides the effects of estrogen levels during pregnancy on breast cancer risk in the offspring, several other candidate mechanisms have been put forward that may underly the relationship between diet during pregnancy and breast cancer in the offspring.

Firstly, a high intake of n-6 PUFAs (of which linoleic acid is the most abundant) which is associated with an increased breast cancer risk (see par. 2.1), leads to an increased metabolic conversion of linoleic acid. This will lead to increased arachidonic acid levels in the mammary gland. This may alter estrogen's signal transduction pathways as well as other estrogen-regulated genes. For example, arachidonic acid (n-6 PUFA) activates a P450 aromatase that causes an increase in the production of estrone, which can be converted to estradiol by adipose tissue [34]. Moreover, a higher arachidonic acid concentration also leads to higher levels of the eicosanoids which are formed from arachidonic acid in the cyclooxygenase pathway (prostaglandin's, thromboxanes, prostacyclins) and in the lipoxygenase pathways (of which the most important one is 12-hydroxyeicosatetraenoic 12-HETE). Inhibitors of prostaglandin synthesis have been shown to counteract the effects of n-6 fatty acids on transplantable mouse mammary tumors and human breast cancer cell lines [35;36]. A correlation has been found between a high dietary intake of n-6 fatty acids and 12-HETE levels (a product of the lipoxygenase pathway) in human mammary tumors growing in nude mice [37]. Metabolic conversion products of arachidonic acid may therefore also play a role in mediating the effects of n-6 fatty acids on breast cancer risk.

A second mechanism for the association of diet during pregnancy and breast cancer risk in the offspring is that the fat content of the diet may play a role per se. A high-fat diet may have induced changes in the structural composition of membranes [38] or in metabolic pathways. For example, protein kinase C, which plays a role in signal transduction pathways, seems to be altered with a high-fat diet or a diet high in linoleic acid [30,39].

A third mechanism in the relationship between maternal diet and breast cancer risk in the offspring is the possible involvement of lipid peroxidation.It is known that unsaturated bonds in the fatty acid are prone to free-radical initiated lipid peroxidation and that at least two unsaturated bonds are required for this process to occur. This is suggested to be one of the mechanisms in explaining the observed modest protection of olive oil against breast cancer [42], which consists mainly of the monounsaturated fatty acid oleic acid. Unfortunately, no studies relating monounsaturated fatty acid intake during pregnancy and breast cancer risk in the offspring have been reported.

# 3. Programming of cardiovascular disease

Cardiovascular disease is the most important cause of death in the Netherlands [43]. Genetic susceptibility and life-style factors both play a role in determining the risk of cardiovascular disease. In addition, evidence is accumulating for a relationship between maternal diet, pregnancy outcome (e.g. growth and size parameters) and risk of cardiovascular disease in the offspring. The research group of Barker (Southampton, England) were pioneers in this area and have performed a large number of epidemiological studies relating parameters at birth (birth weight, thinness at birth) to risk of cardiovascular disease in adulthood. The hypothesis that individuals who are small at birth are at increased risk of developing cardiovascular disease in adulthood is therefore also referred to as the 'Barker hypothesis'.
In the first part of this chapter, we will focus on the relationship between birth weight and cardiovascular disease. Diseases studied within this context include ischaemic heart disease (coronary failure, cardiac arrest) and cerebrovascular diseases (stroke). The role of maternal diet in the determination of birth weight is discussed and the effect of (catch-up) growth and body composition in childhood on cardiovascular risk is described.
Hypertension, an important risk factor for cardiovascular disease, is considered in the second part of this chapter. The role of maternal diet and obesity in relation to blood pressure in the offspring as well as possible underlying mechanisms are discussed. Figure 2 summarizes the factors implicated in the relationship between diet during pregnancy and cardiovascular disease in the offspring in adulthood.

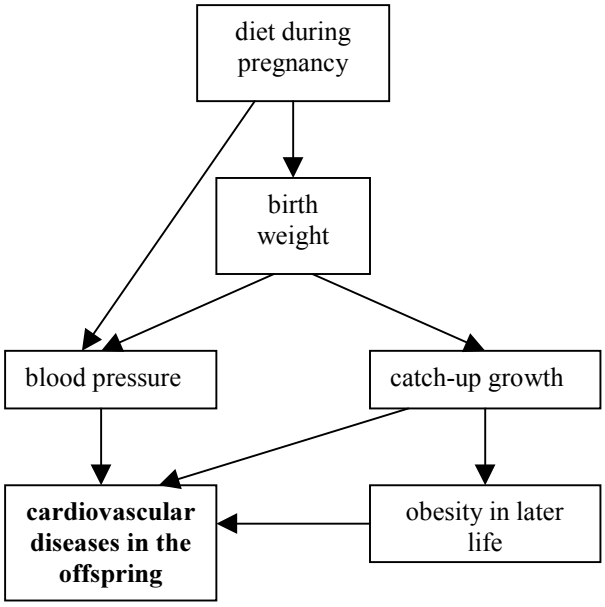


*Figure 2: Interrelationship of factors implicated in prenatal nutritional programming of cardiovascular diseases.*

## 3.1 Size and growth in early life

A number of specific lines of evidence now support the idea that small babies with a low birth weight are at greater risk for developing cardiovascular diseases in adulthood. Studies of the birth weights of relatives [44], together with evidence from animal cross breeding experiments [45] have led to the conclusion that the diversity in body size and proportions at birth is essentially determined by the intrauterine environment rather than the fetal genome [46]. In this regard, the maternal supply of nutrients and oxygen seems to be the limiting factor for fetal growth [47].

The simplest way of adapting to undernutrition is to reduce growth, thereby reducing the use of substrates. When the nutritional need of the fetus is not covered by the maternal supply, the placenta becomes enlarged relative to the size of the fetus to compensate for the lower substrate supply. A low birth weight and disproportion's in head circumference, length, weight and placental weight, reflect therefore adaptations which the fetus made to sustain its development [48].

A series of parameters are currently being used in studies to describe the size and growth of the fetus. A well-known crude parameter of fetal development is birth weight, which is a combination of head size, body length, and fatness. An other parameter is 'small for gestational age' (SGA), indicating that the weight relative to the gestational age of the baby is below a given percentile. However, babies who are SGA need not be growth retarded *in utero*: some babies are 'genetically small'. Intrauterine growth retardation (IUGR) is diagnosed by serial measurements of anthropometric parameters throughout pregnancy and by comparing the growth trajectory with population-based growth charts developed from longitudinal studies [49]. One parameter to indicate IUGR is ponderal index, which is the weight of the baby divided by the cube of its length.Two patterns of IUGR are commonly described. The first one is the symmetric growth retardation: the fetus is small but with normal proportions. This pattern of growth retardation is assumed to result from long-term intrauterine growth impairment, i.e. the factors causing the growth retardation have exerted their effects from the second trimester of pregnancy. The other pattern of impaired fetal growth is the asymmetric one. In this case fetuses are characterised by small abdominal circumference due to a small liver, decreased abdominal and subcutaneous fat and reduced mass of skeletal muscles. The size of the head is often in the normal range. Asymmetric body proportions are assumed to result from impaired growth restricted to the last trimester of pregnancy, caused by e.g. short-term malnutrition [49]. Retrospective studies have demonstrated that asymmetrical growth retardation of the human fetus is a strong predictor of later hypertension, hypercholesterolaemia and death from coronary heart disease [50;51].

### 3.1.1 Fetal growth, birth weight and cardiovascular disease risk: epidemiology

A series of epidemiological studies have shown an inverse association between birth weight and the incidence of cardiovascular disease in adulthood. Barker et al. found a relationship between the geographical pattern of death rates among babies in Britain during the early 1900s and today's pattern in death rates from coronary heart disease (CHD)[52]. One possible conclusion was that low rates of growth before birth are in some way linked to the development of coronary heart disease in adult life.

When the birth records of 16,000 men and women born in Hertfordshire (1911-1930) were related to examination records of the same men and women in their fifties and sixties it became clear that death rates from coronary heart disease increased 2-fold for those in the lowest percentile of the birth weight distribution compared to those in the highest percentile [53]. This same picture has emerged from an analysis of cardiovascular disease (CVD) in the US among 80,000 women in the Nurses Health Study, in which also a 2-fold fall in relative risk of CVD was present with increasing birth weight [54]. Also in South India, this same association between low birth weight and prevalent coronary heart disease has

recently been observed [155.] Among the men and women in this study, the prevalence of the disease fell from 15% in those who weighed 2.5 kg at birth to 4% in those who weighed 3.2 kg or more.

A study in Sheffield [55] among 1586 men showed that reduced fetal growth (expressed as a small head circumference, thinness at birth and a high ratio of placental weight to birth weight) was followed by higher death rates from CVD in adult life. An other large cohort study in Uppsala, Sweden [56] (14 611 subjects) also showed the inverse relationship between birth weight and mortality for CVD until age 65 (p-value for trend: <0.001). However, birth weight is a function of gestational age at delivery and of fetal growth rate. When the effect of birth weight on CVD mortality was separated from that of fetal growth (measured as birth weight for gestational age), the effect of birth weight was almost entirely eliminated, while the effect of birth weight for gestational age was slightly strengthened. They concluded that it was the rate of fetal growth that underlied the association of birth weight with CVD mortality.

In two large epidemiological studies in the U.K. [57;58], men and women who had a low birth weight were not only at an increased risk for coronary heart disease, but the levels of cardiovascular risk factors which are associated with insulin resistance were also altered. The subjects showed abnormal glucose and insulin metabolism, raised serum triglyceride concentrations, low serum high-density lipoprotein cholesterol concentrations and a high waist:hip ratio. These findings indicate that type II diabetes and hypertension may have a common origin in sub-optimal development *in utero*. It should be noted that in all studies, the strong and graded associations between birth weight and cardiovascular disease were independent of social class (at birth or currently), and were also independent of influences such as smoking, obesity and alcohol consumption in later life [48;56;59-61].

## 3.1.2 Birth weight and blood pressure during adulthood

Hypertension (high blood pressure) is an important risk factor for the development of CVD. The relation between birth weight and blood pressure is possibly the best-studied association between fetal growth and later physiological characteristics. Law and Shiell [62] examined the results of 32 papers identified in a systematic search of the literature. This review shows clearly that at all ages, with the possible exception of the pubertal period, there is an inverse relationship between blood pressure and birth weight. This association is seen in different countries and in males and females. Socio-economic factors do not provide an explanation for the association between birth weight and blood pressure [63]. In general it has been found that adjustment for weight or body mass index (BMI) at the time at which blood pressure is measured increases the strength of the association [63]. Most studies have been criticised for being based on ill-defined populations, for the large numbers of subjects who were unavailable for follow-up, and for inadequate control of socio-economic status. Many of these criticisms were addressed in a prospective observational study by Klebanoff et al. [64]. Their results supported the finding that women who were small for gestational age (SGA) at birth had an increased risk of developing a variety of risk factors for cardiovascular disease including hypertension during adulthood. They were also at increased risk of developing hypertension during pregnancy.
Despite the consistency of the findings, some (parental) confounders (maternal smoking, maternal blood pressure) in the relationship between birth weight and adult blood pressure may be present. Poulter et al. [65] compared 492 pairs of female twins and found that the inverse association between birth weight and blood pressure is independent of parental variables (weight, height, smoking, alcohol consumption).

The contribution of parental blood pressures to the relation between low birth weight and subsequent hypertension was studied by Walker et al. [66] in a cross-sectional study. It was found that mothers with higher blood pressure measured between 9 and 19 years after their pregnancy had offspring with a lower birth weight, who also developed higher blood pressure. These results suggest that low birth weight may be, at least in part, a feature of the inherited contribution to hypertension, perhaps because it is associated with higher maternal blood pressure during pregnancy. In this context, the inherited contribution may be mediated by genetic or by shared environmental factors or both.

Therefore, the correlation between low birth weight and subsequent high blood pressure seems to be confounded by the influence of parental blood pressure. However, birth weight was not related to paternal blood pressure. This may be because of the possibly more important influence of maternal size on body weight and the putative imprinting of exclusively maternal genes [67].
In a cohort study among children from multiple pregnancies (multiplets), mostly twins, Dwyer et al. [68] studied the association between birth weight and blood pressure at age 8 which was stronger in multiplets than in singletons, suggesting that shared environmental factors *in utero* may play a role in this association.The association was not weakened in within pair analysis and also remained within monozygotic pairs, suggesting that the association between birth weight and blood pressure does not originate in the genes of the infants. The authors suggest that important causes of the association seem to be operating within the fetoplacental unit. For example, the supply of oxygen and nutrients across the placenta can be unequally distributed between individual multiplets and may be more prevalent causes of growth restriction in multiplets than in singletons. In this way, this may lead to permanent adverse programming of the cardiovascular system in multiplets.

### 3.1.3 Maternal diet and birth weight

Maternal weight gain during pregnancy is regularly monitored for several reasons. Maternal weight gain and infant birth weight are modulated by maternal food consumption. A variety of studies have addressed the relationship between diet in pregnancy and birth weight, which are summarised in Table 1.

The Dutch Famine study [69] illustrates the above statements, but only under the conditions of severe famine. The Dutch famine (November 1944 until May 1945) had a sudden beginning and end, and struck the entire population in certain geographic areas, irrespective of social class. During this period, caloric intakes declined sharply and to very low levels at the extreme (~500 kcal/d). After some delay, a sharp decline in maternal weight followed the caloric decline. No decrease in birth weight in the offspring of women exposed to undernutrition in early pregnancy (first trimester) could be found [70-72] whereas birth weight of children of women exposed to undernutrition in late pregnancy (third trimester) was decreased. Birth weights fell about ~300 g in the cohort with the third-trimester exposure. It was concluded that the correlation of birth weight with maternal weight held below a dietary threshold only, which suggests that birth weight was mediated through maternal weight but only below that threshold [73]. Tentative support has been found in one other study, under the condition of chronic nutritional deprivation and only if the fetus is male [74]. Five other studies under other conditions are not supportive [142-150]. Therefore, diet effects on birth weight apparently bypass maternal weight change in non-famine conditions [73].

The question of whether the quality of the diet rather than the quantity affects birth weight was recently studied by Matthew's et al.[75] in a large and detailed study. They investigated the relations of maternal diet during pregnancy with placental and birth weight. Women (n=693) recorded their food intake with a 7 day food diary and usual diet was measured with a food frequency questionnaire at 28 weeks. Vitamin C intake in early pregnancy (first trimester) but no other nutrient, was positively related to birth weight, with about a 100 g difference between the lowest and highest thirds of intake. The significance of this relation was however considerably reduced after adjustment for smoking and maternal height (p-value of 0.002 before vs 0.031 after adjustment). Vitamin C intake in early pregnancy showed a weak correlation with placental weight after adjustment for maternal height. As in previous research [76-78], this study found no association between any nutrient in later pregnancy and placental or birth weight. An other prospective study of dietary factors in pregnancy and fetal growth and birth weight [79] showed that riboflavin (vitamin B2) was positively associated with birth weight and length (1 mg riboflavin was associated with an increase in birth length of almost 1 cm and with an increase in birth weight of about 149 gram). A negative relationship was observed between head circumference of the newborn and maternal intake of linoleic acid intake. It is known that increasing linoleic acid levels suppress the incorporation of n-3 fatty acids in phospholipids, particularly the n-3 fatty acid DHA (docosahexaenoic acid). As DHA is an important fatty acid for brain development,

this may explain the negative relationship between maternal linoleic acid intake and neonatal head circumference [79]. Olsen et al.[154] reviewed the current epidemiological evidence relating maternal n-3 fatty acid intake during pregnancy as a possible determinant of birth weight. He concluded that marine n-3 fatty acids increase birth weight when ingested in late pregnancy via a prolonging of the pregnancy duration.

With respect to carbohydrates and protein, Barker et al. [77] found that a high carbohydrate intake in early pregnancy was associated with low placental and birth weights (49 g vs 165 g decrease respectively for each log g increase in carbohydrate intake). However, low maternal intakes of dairy and meat protein in late pregnancy were associated with lower placental and birth weights (placental weight: 1,4 g decrease for each g decrease of dairy protein intake; birth weight: 3,1 g decrease for each g decrease of meat protein intake), suggesting that carbohydrate and protein may have different effects in early and late pregnancy. These associations persisted after adjustment for maternal height, BMI and of relations between the mother's birth weight and the placental and birth weight of her offspring.

Kramer et al. [80] reviewed the research evidence from controlled clinical trials for the effects of energy and protein supplementation during the entire pregnancy on the outcome of pregnancy (birth weight, length, head circumference). They concluded that a balanced increase in energy and protein intake during pregnancy in women with an adequate nutritional status results in modest increases in maternal weight gain and fetal growth. Surprisingly, these increases did not appear to be larger in undernourished women, nor did they seem to confer long-term benefits to the child in terms of growth or neurocognitive development. The modest benefits of energy and protein supplementation may be explained by the rather modest net increases in energy intake achieved [74;80;81].

*Table 1: Overview of studies, discussed in the text, relating (composition of) maternal diet in different trimesters on pregnancy outcome. Reference numbers are indicated within parenthesis.*

| **pregnancy:** | **0-3 months (first trimester)** | **3-6 months (second trimester)** | **6-9 months (last trimester)** |
|---|---|---|---|
| undernutrition (caloric decline) of the mother: | no decrease in birth weight (70-72) | | decrease in birth weight ~300 g (70-72) |
| quality/composition of maternal diet: | | | |
| *vitamin C intake* | positive association with birth weight (75) | | |
| *vitamin B2 intake* | positive association with birth weight and birth length (79) | positive association with birth weight and birth length (79) | positive association with birth weight and birth length (79) |
| *linoleic acid intake* | negative association with head circumference (79) | | |
| *N-3 PUFAs + arachidonic acid* | positive association with birth length (79) | positive association with birth length (79) | - positive association with birth length (79)<br>- positive association with birth weight (154) |
| *other nutrients* | | | no association with birth weight/placental weight (75-78) |
| *carbohydrate intake* | negative association with birth weight and placental weight (77) | | |
| *intake of dairy/meat protein* | | | negative association with birth weight /placental weight (77) |
| *energy and protein supplementation* | positive association with fetal growth (80) | positive association with fetal growth (80) | positive association with fetal growth (80) |

Other factors which have been shown to influence birth weight include gestational age and sex of the child, maternal height and weight, ethnicity [82] and maternal age [83]. A well-controlled study by Wilcox [82] has shown an association of birth weight with socio-economic status, whereas a study by Brooke et al. [84] showed no effect of social variables. An additional factor found to play a role in the determination of birth weight of the fetus is the birth weight of the mother herself. Mothers of growth-retarded babies had themselves a low mean birth weight [85]. Also Emanuel et al. [86;87] and Magnus et al. [88] showed that the birth weight of the next generation is associated with maternal birth weight. However, the Dutch Famine study did not show any long-term consequences for offspring birth weight patterns in women who were exposed *in utero* to undernutrition in the third trimester (and had a profound decrease in birth weight). Other studies have shown that the birth weight of mothers is not only related to that of their children but also to that of the subsequent generation[89;90]. It is currently thought [156] that the mother has a certain limited capacity to deliver nutrients to her fetus and that this is determined by the size of the uterus, the nutritional status of the mother at the onset of pregnancy and even the nutritional status of the mothers own mother in the past.

One recent study argues against a major role for intrauterine nutritional deprivation as a cause for the association between birth weight and subsequent adult disease. Several studies have reported significant differences in concentrations of nutritional metabolites between low and normal birth weight for gestational age fetuses [91-95]. Low birth weight babies are more often hypoglycaemic, hypoinsulinaemic, hypertriglyceridaemic and hypercholesterolaemic compared with those of normal size [91-95]. Spencer et al. [96] showed that there is no difference in carbohydrate and lipid metabolism in umbilical venous blood from small term babies who were growth-retarded in the third trimester (when the increase in the size of the foetus is the largest) compared to 'genetically' small babies (without growth retardation) [96]. It is concluded that the similar lipoprotein, triglyceride and cholesterol profiles do not provide substantial evidence of a predisposition to atherogenesis as a result of reduced fetal growth. The authors suggest that it is more likely that the epidemiological association found between birth weight and subsequent adult disease reflects familial and genetic tendencies rather than interference with fetal nutrition during the third trimester of pregnancy.

### 3.1.4 Catch-up growth and cardiovascular disease risk

Recent evidence has been found in cohort studies [97] for a role of 'catch-up' growth in modulating the intrauterine programmed effects. Catch-up growth is defined as the crossing of at least one percentile in standard growth charts and is seen mainly in the first 1-2 years of life [152] . By 2 years of age, growth usually follows the genetic trajectory. These variable growth rates often compensate for intrauterine restraint of fetal growth.

In a large longitudinal study of 3641 men and 3447 women [97;98] in Helsinki, it was investigated whether growth in childhood could modify the effect of reduced prenatal growth. With respect to size at birth and cardiovascular risk, it was found that coronary heart disease is associated stronger with short body length in women and thinness in men than with birth weight in this cohort. The patterns of fetal growth among women and men in this cohort differed whereby female fetuses grew more slowly. The authors suggest this may explain a part of the lower rates of coronary heart disease seen in women compared to men. In both men and women the effects of reduced fetal growth on the risk of coronary heart disease were increased by accelerated or 'catch-up' growth in childhood (measurements at age 7, 11 and 15). Among women, those who were small at birth but tall in childhood had the highest risk of developing coronary heart disease [98]. In addition, the men who were thin at birth but had an above average body mass index in childhood were at increased risk [97]. In men, the risk of coronary heart disease was not influenced by height in childhood whereas in women no effect of body mass in childhood on cardiovascular risk could be found. It is concluded that coronary heart disease among both men and women reflects poor prenatal nutrition and consequent small body size at birth combined with improved postnatal nutrition and 'catch up' growth in childhood.

Ong et al.[152] identified predictors of catch-up growth and studied the relationship between postnatal catch-up growth and obesity in childhood among 848 healthy singletons in the United Kingdom. The children who showed catch-up growth during the first two years of life (30.7%) were lighter and thinner at birth than other children and their mothers had lower birth weights and smoked more often, indicating that fetal growth in these children had been restrained. Children who showed catch-up growth had taller fathers than the other children. This may indicate a postnatal effect of paternal genes on childhood growth but could also be explained by increased maternal restraint in response to greater fetal growth potential. The children who showed catch-up growth during the first two years of life were heavier and taller at five years of age than other children of the same age and they also had a greater body mass index (weight/length$^2$), percentage body fat, total fat mass and central fat distribution, which are parameters linked to metabolic markers for risk of disease in adulthood and which are predictive for adulthood obesity.
How infants who were restrained in utero catch up postnatally is largely unknown, although greater food intake has been observed compared with other infants [152]. Ong et al.[152] state that since concentrations of cord blood leptin are positively related to ponderal index at birth but inversely related to weight gain in infancy, low concentrations of leptin at birth may provide a signal for catch-up growth through reduced inhibition of satiety.

It has been suggested that catch-up growth could be deleterious through overgrowth of a limited (fat) cell mass, which would disrupt cell function [98,99]. In addition, restricted fetal growth may lead to relatively reduced cell numbers in organs such as the kidney, in which there is no further cell replication after birth [99]. A large body size in later life imposes an excessive metabolic demand on this limited cell mass [98].

In summary, an inverse association between birth weight and risk for cardiovascular diseases has been confirmed in many epidemiological studies. Moreover, the pattern of fetal growth seems to be more important than birth weight per se in determining cardiovascular risk. No consistent results have been found with respect to the composition of maternal diet and birth weight of the offspring.

Recently, postnatal growth and nutrition have been suggested to modify the effects of intrauterine programming. Poor prenatal nutrition combined with improved postnatal nutrition and 'catch-up' growth in childhood seems to be associated with the greatest risk of cardiovascular diseases in men and women.

## 3.2 Maternal diet, birth weight and blood pressure in adulthood

Blood pressure is an important risk factor for cardiovascular disease. Evidence for a relationship between diet during pregnancy and hypertension in the offspring will be discussed in this section. In particular, the role of amino acids in determining fetal growth and birth weight will be outlined.

### 3.2.1 Maternal diet and blood pressure in the offspring

With respect to the effects of maternal diet on blood pressure in adulthood, the Dutch Famine (1944) was a natural experiment. During this famine, the caloric content of the diet decreased strongly (to 600-800 kcal) but the balance between protein, carbohydrate and fat remained approximately the same. No effect of this balanced reduction of macro-nutrients on blood pressure in the offspring could be found, and Roseboom et al.[100] postulate that it might be the composition rather than the quantity of a pregnant woman's diet that affects her child's blood pressure in later life.
Considering changes in individual macro-nutrients, a retrospective study in Aberdeen among 253 men showed that in the presence of a daily maternal animal protein intake below 50 g, a high carbohydrate intake was associated with higher offspring systolic blood pressures 40 to 50 years later (~3 mm Hg increase in systolic blood pressure per 100 g increase in carbohydrate intake) [76]. However, when the protein intake exceeded 50 g daily, women with a low carbohydrate intake had offspring with raised blood pressure, indicating that the balance between macronutrient intakes rather than the absolute level of intake is related to blood pressure.

The effect of the protein content of the maternal diet on the programming of blood pressure in the offspring has been confirmed in animal studies. McCarty et al. [101] used the Spontaneously Hypertensive Rat (SHR) to determine the timing of preweaning maternal influences on the development of high blood pressure. In the SHR mouse, hypertension is being regarded as of purely genetic origin. Cross-fostering of SHR offspring with normotensive dams during the early postnatal period prevented the development of hypertension compared to pups that were fed by SHR mothers [101]. The reverse experiment, in which normotensive pups were cross-fostered to SHR mothers did not result in hypertension [102]. Analysis of the SHR milk showed that the overall protein content is markedly lower, the balance of electrolytes is different and the profile of polyunsaturated fatty acids is altered. In several animal experiments, the protein content of the maternal diet was manipulated to study the magnitude of the effects on blood pressure of the offspring. Langley-Evans et al. [103] exposed

pregnant rats to a diet containing 18% (control), 12%, 9% or 6% casein. Exposure to a 9% casein diet appeared to accelerate fetal growth (day 14 – day 20) but at birth, the offspring were of low to normal birth weight. The rat's fed 6% casein failed to grow between day 20 and term and also their postnatal growth was permanently impaired compared to 18% casein-exposed animals. The tissues of rats exposed to low-protein diets in utero were biochemically and anatomically different from those of the offspring of well-nourished rats. The kidneys were typically small in proportion to body weight, the liver exhibited evidence of a permanently altered function and the structure and function of the endocrine pancreas was disturbed. The low-protein fed rats developed a raised systolic blood pressure from 4 weeks of age onwards and this effect appeared to be persistent and probably lifelong. Interestingly, Reusens et al. [104] found that a isocaloric, protein-restriced diet given to rat dams, also affected weight gain in the fetus. A significant reduction in birth weight was found between the low-protein newborns versus in the control newborns. Clarke et al. [105] showed that even a 12% casein diet, which just meets the requirements for protein provision in rat pregnancy, produced a similar blood pressure increase to that elicited by a profound nutritional insult (6% casein diet). The 12% casein diet also produced placental enlargement and retardation of fetal growth.

Interactions between protein content and type of fatty acids in the diet during pregnancy as regards effects on the blood pressure in the offspring have been studied by Langley-Evans [106]. Diet groups with varying protein content and fat sources were defined. Protein content as well as fat source in the diet influenced the blood pressure in the offspring. The observed hypertensive action of coconut oil may be attributable to either the higher saturated fatty acid content of the diet or the lower linoleic acid content. The latter is especially of interest with regard to blood pressure changes, as linoleic acid is known to have hypotensive effects in animals [107]. Furthermore, the rats fed coconut diet had lower birth weights and body weights (due to a lower food intake) and smaller livers (as percentage of body weight) compared to control rats, indicative of prenatal programming of metabolic activity [107].
In an other study, Langley-Evans [108] confirmed the hypertensive effects of the coconut oil diet and the hypotensive effect of the corn oil diet in the offspring when fed during pregnancy. However, in animals exposed in utero to a 9% casein diet, coconut oil or corn oil diets given from 7 weeks of age onward had no effect on blood pressure, suggesting that prenatal diet may modulate the effects of fatty acids consumed postnatally.

### 3.2.2 Mechanisms of programming of adult blood pressure

The mechanism of programming of blood pressure by maternal diet appears to relate to fetal growth, amino acids, glucocorticoids and the fetal adrenal.
Protein restriction (9% casein) has been shown to accelerate growth of the rat fetus from mid-gestation until late gestation (Langley-Evans et al.[110] ). However, fetal growth retardation and an enlargement of the liver of the rat at day 20 of gestation has been shown on the same protein restricted diet in an other study of the same research group [111]. A larger methionine supplement was given in the first study, suggesting that the growth retardation up to day 20 may be, at least in part, attributable to the effects of a sulfur-limiting diet. The accelerated growth from mid- to late gestation suggests that an adaptive response in the fetus or placenta allows a more efficient transfer of substrates from mother to fetus, or a more effective utilization of substrate, when amino acids are limiting. This adaptation in the fetus may be achieved through changes in the expression of the insulin-like growth factors (IGFs) and their binding proteins. IGF-I is an important regulating factor influencing the distribution of substrates between maternal tissues, fetal tissues, and the placenta. Exposure of rats to a protein deficient diet or to a low calorie diet lowers IGF-1 expression [112;113]. Also the fetal and placental weights in late gestation are strongly related to serum IGF-I and IGF-II concentrations in sheep and other species [112;114]. In human infants, plasma IGF-1 levels correlate with both existing blood pressure and the preceding birth weight [115]. The detailed biochemical and molecular mechanisms remain to be identified [2]. Of interest is that placental function may control IGF expression and hence modulate fetal growth through regulation of substrate utilisation.

A role of specific amino acids on fetal growth during pregnancy has been suggested [116]. Embryonic cells are able to respond to amino acid deficiency by increasing the expression of a variety of genes whose products regulate growth, differentiation and apoptosis [117]. Rees et al.[138] showed in an animal experiment that, whilst a reduction in the protein content of the maternal diet does not produce a generalised reduction in the amino acids, there was a 75% decrease in free threonine on day 19 in both mother and fetuses when the pregnant animals were fed on low-protein diets. Control animals fed 18% casein showed a 50% fall in serum threonine concentrations. The changes in threonine concentrations occurred only in the pregnant animals indicating that this level of protein (9% casein) was therefore not deficient with regard to threonine requirements of the non-pregnant animal fed 9% casein. On day 19, the fetuses of the 9% casein group were approximately 7.5% heavier than the pregnant animals fed 18% casein. The weights of the kidneys were increased in the 9% casein group when expressed as a percentage of the total fetal weight. However, on day 21, the fetuses in the 9% casein group were 14% lighter than those of the pregnant rats in the 18% casein group. Expressed as a proportion of the total fetal weight, the liver was a significantly smaller part of the whole fetus whereas the heart and brain were larger. The placentas were also significantly smaller in the group fed the 9% casein diet. An increase of nearly 2-fold in free glycine concentrations was observed in the rats fed the low protein diet. Since deamination of threonine leads to glycine production, the fall in circulating threonine concentrations may be due to its metabolism to produce glycine. Although often considered as non-essential, glycine may become essential during pregnancy and is considered to be potentially limiting during pregnancy in human subjects [140] . Glycine is important in the synthesis of many compounds required for growth, both structural and functional, e.g. collagen, haem, bile salts and glutathione [141.] An other amino acid that has been shown to change significantly in the serum of pregnant rats fed a low protein diet is taurine [104]. Taurine is known to play an essential role in brain development, constitutes over 50% of the free amino acids in many tissues and may play a key role during development as well as in later life. In humans, alterations in taurine concentrations in tissues as well as in plasma have been detected in hypertensive patients. Taurine concentrations are also very low in the breast milk of vegans which shows that taurine metabolism is indeed modulated by the reduction of total protein intake [104].

In the low protein models of programming, it was observed that the rats who developed high blood pressure had a 33% lower placental 11 β-hydroxysteroid dehydrogenase (11 β-HSD) activity [139]. This enzyme precludes access of glucocorticoids from the maternal circulation to the fetal tissues, in rats as well as in humans, by converting active maternal cortisol into inactive metabolites. Benediktsson et al. in 1993 [118]hypothesized that an overexposure of the fetus to maternal glucocorticoids because of a low 11 β-HSD activity causes a permanent resetting of the function of the fetal hypothalamic-pituitary-adrenal (HPA) axis. Studies in the rat have demonstrated that the activity of placental 11 β-HSD is directly proportional to fetal weight and inversely related to placental weight [118]. Similar relationships are seen in human pregnancy, where 11 β-HSD activity in full term placenta is correlated with birth weight [119] but not with placental weight. These data indicate that in those individuals perceived to be at greatest risk of cardiovascular disease, i.e. those of low birth weight with a large placenta, protection against exposure to maternal glucocorticoids is lowest. Animals exposed to carbenoxolone, which is an inhibitor of 11 β-HSD, are smaller at birth and have significantly raised blood pressures [118]. The effect of carbenoxolone is dependent upon an intact maternal adrenal, suggesting it is mediated by glucocorticoids of maternal origin. There is also evidence in humans that exposure to synthetic glucocorticoids can retard growth of human fetuses [120] and can increase blood pressure in adult humans [151]. A maternal protein restriction possibly attenuates the activity of placental 11 β-HSD and this increased exposure to glucocorticoids may alter fetal blood pressure. In addition, the placental activity of the enzyme may play a critical role in the development of the fetal adrenal and hence may determine patterns of glucocorticoid secretion throughout life. In the short term, the increase in fetal steroid exposure may be of advantage to the fetus, providing accelerated maturation of key organs such as the lung. In the long term, the steroid hormones may exert more deleterious effects in for example the kidney.

An alternative candidate mechanism whereby adult hypertension is programmed *in utero* is mediated by the fetal kidney. The offspring of animals fed a low protein diet during pregnancy have small kidneys in proportion to body mass. They also appear to have fewer glomeruli in adult life [110]. Lower

numbers of glomerula have also been observed in growth retarded human infants and appear to result in impaired renal function [121].

It appears that glucocorticoids, the kidney and the renin-angiotensin system are all interrelated with respect to blood pressure control. In figure 3, the candidate mechanisms of the programming of hypertension *in utero* are given.

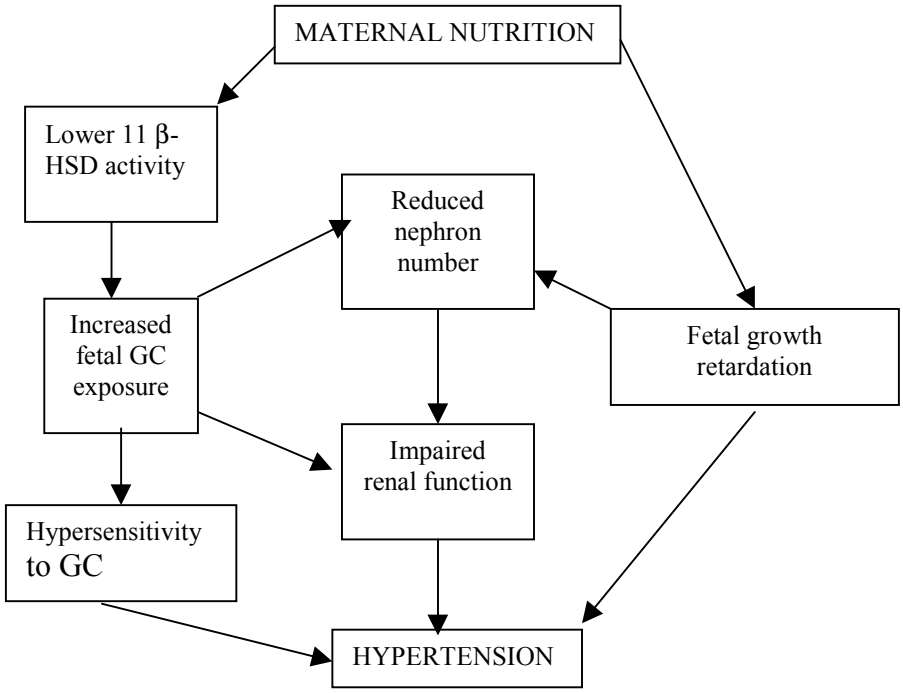


*Figure 3: The fetal origins of hypertension: the working hypothesis. (From: Langley Evans SC, D. Gardner DS, and Welham SJ. Intrauterine programming of cardiovascular disease by maternal nutritional status. Nutrition. 14 (1):39-47, 1998) GC= glucocorticoid*

# 4. Programming of non-insulin dependent diabetes mellitus

There exists a well-known association between cardiovascular disease (CVD), hypertension and non-insulin dependent diabetes mellitus (NIDDM). Evidence has also been found for a role of poor intrauterine nutrition in determining the susceptibility of NIDDM in the offspring. However, the role of genetic predisposition as a factor determing risk of NIDDM is also important. These two relations, which both may explain a part of small thin babies having a higher risk of NIDDM, are depicted in figure 4. In this chapter, the effect of maternal diet on the developing and growing endocrine pancreas and the consequences for the susceptibility to NIDDM in the offspring will be discussed.

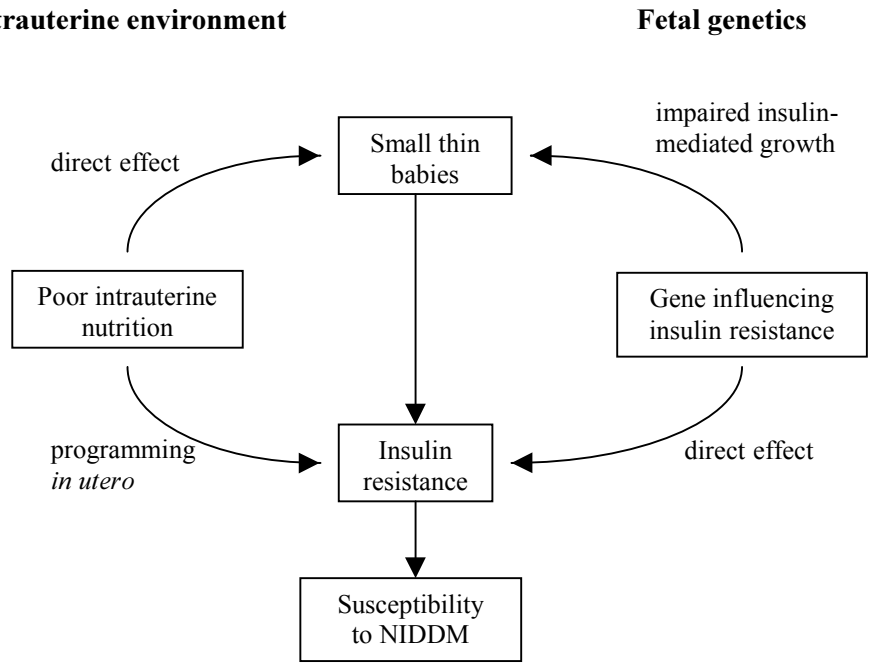


*Figure 4: Two alternative explanations for association of small, thin babies with insulin resistance and non-insulin dependent diabetes mellitus (NIDDM)– intrauterine environment and fetal genetics. (Modified from: Hattersley AT, Tooke JE, Lancet; 353: 1789-92, 1999)*

# 4.1 Maternal diet and the endocrine pancreas

Epidemiological studies have shown associations between pregnancy outcome and the risk of developing insulin resistance or diabetes in adulthood. In the Hertfordshire study (1995), the percentage of men with impaired glucose tolerance (measured by a full 75 g oral glucose tolerance test) or type II diabetes mellitus fell progressively with increasing birth weight and weight at one year [122]. Although there is evidence that gestational diabetes predisposes to diabetes in the offspring, this could not explain the finding that the largest babies are those least likely to develop diabetes. Unlike glucose tolerance, the blood pressure in the Hertfordshire men was inversely related to birth weight but not to weight at one year. Barker et al. hypothesise that factors affecting early growth may therefore lead to either high blood pressure or impaired glucose tolerance/type II diabetes, depending on the exact timing of the growth impairment during fetal or infant life. Barker et al. (1992) proposed that poor maternal nutrition causes reduced fetal growth and later glucose intolerance and that this effect is mediated through impairment of β-cell function. Growth retarded newborn infants have indeed been shown to have reduced numbers of β-cells and a reduced insulin secretion [123].

Exposure of experimental animals to general protein/calorie malnutrition or to a protein deficient diet alone caused a reduction in the number and size of pancreatic islet cells and reduced the vascularization of the endocrine pancreas [1;104;124-127] in the offspring at birth. Reusens et al.[104] showed that a normal diet postnatally does not completely restore the lesions induced by a low-protein diet during pregnancy in the offspring. Also in humans, the low birth weight progeny of mothers consuming an isocaloric, low-protein diet during pregnancy, are unable to regain an appropriate insulin secretory response to a glucose challenge even when given a normal protein diet throughout postnatal life [128].
Observations from the Preston study [129] have led to the suggestion that the association between fetal growth and glucose intolerance is mediated through insulin resistance rather than through an impaired β-cell function. It was shown that insulin resistance was inversely related to ponderal index (birth weight divided by the cube of the length, a measure of thinness) but not to birth weight. A possible explanation for this observation might be that the thin neonate lacks skeletal muscle, and muscle is the main peripheral site of action of insulin.

In a cohort study in Uppsala (Sweden), Lithell et al. [130] tested whether the relation between size at birth and non-insulin dependent diabetes is mediated through impaired β-cell function or insulin resistance. Of 1333 men whose birth records were traced during 1920-4, the intravenous glucose tolerance at age 50 years and the prevalence of NIDDM at age 60 years was measured. The lack of an association between birth weight and acute insulin response to intravenous glucose challenge in this study and in the earlier Preston study [129] suggests that the relations between reduced fetal growth and diabetes is indeed mediated through insulin resistance rather than through impaired β-cell function. Moreover, the inverse association between ponderal index and insulin resistance and diabetes was strongest in overweight people, suggesting that there is an interaction with obesity in adult life.

It seems that the availability of amino acids to the tissues may be monitored by the fetal β-cell, just as the β-cell senses the availability of nutrients in the adult. Evidence available to date strongly suggests that amino acids are the major factors controlling β-cell growth and development and insulin secretion until late fetal life [123;131]. Fetal insulin secretion is one of the key regulators of intrauterine growth, acting mainly in the third trimester when the weight of the fetus increases greatly. The role of fetal insulin and fetal genetic factors has been shown in figure 4 as an alternative explanation for the association between birth weight and diabetes and CVD. The important role of insulin and glucose during pregnancy on fetal growth has led Barker et al. to propose the 'thrifty phenotype hypothesis' (1992, figure 5). The essence of this hypothesis is that poor nutrition (a thrifty situation) in fetal and early infant life is detrimental to the development and function of the β-cells of the islets of Langerhans. As long as the individual persists in the undernourished state there is no need to produce much insulin. However, a sudden move to good or overnutrition exposes the reduced state of β-cell function and diabetes results.

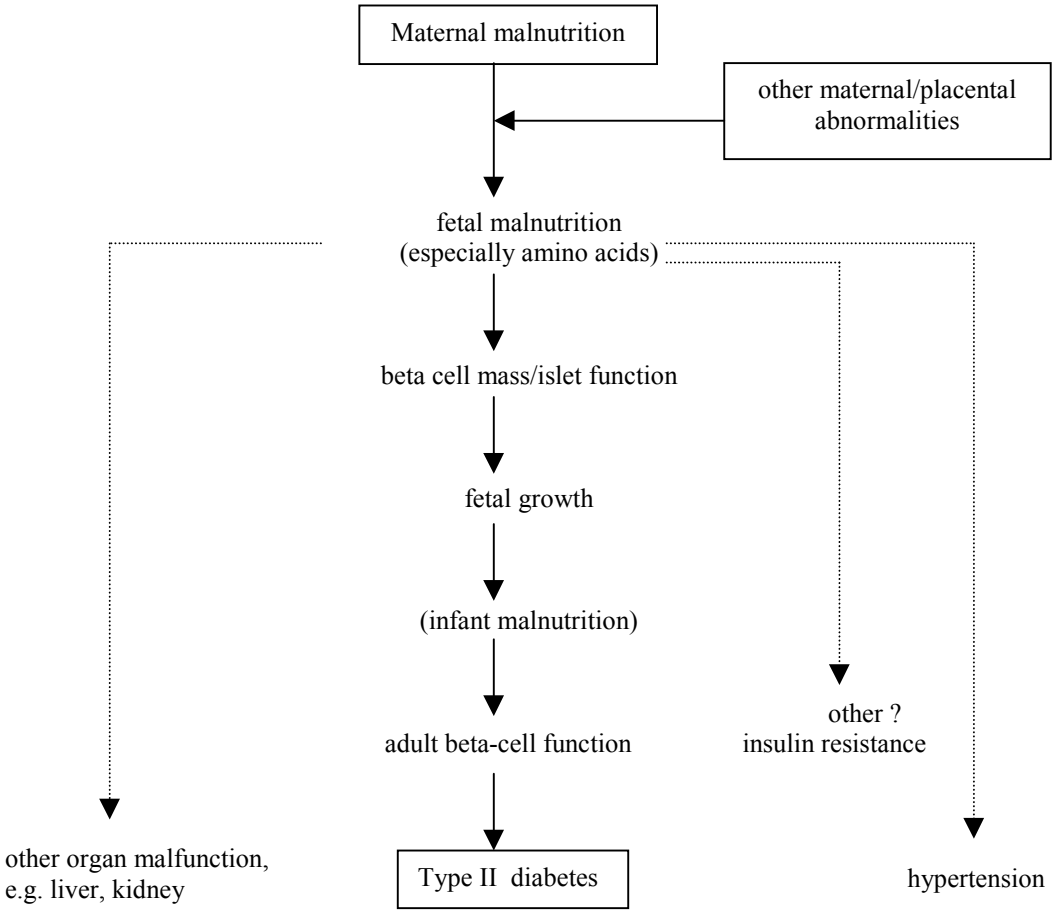


*Figure 5: diagrammatic representation of key features of the 'thrifty phenotype' hypothesis of the aetiology of type II (non-insulin-dependent) diabetes. (From: Hales CN and Barker DJP. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia 35:595-601, 1992)*

In summary, there are suggestions from epidemiological studies as well as from animal experiments that the diet of the mother during pregnancy can have effects on the insulin sensitivity of the

offspring. The maternal protein intake seems to be especially important for determing risk of diabetes in the offspring and this seems to be mediated by insulin resistance rather than impaired β-cell function. Permanent effects of a low protein diet on morphology and on cell numbers of the kidney and pancreas have been shown.

# 5. Programming of infectious disease

Prenatal or early postnatal events may affect the future susceptibility to infections. This is illustrated by a study of Moore et al. [132], who studied the relationship between seasonality in Gambia and the mortality in 3,102 individuals. In Gambia, the wet season coincides with the annual hungry period and runs from July till October. During this period, adults perform an intensive agricultural workload and infectious diseases like diarrhoea and malaria are rampant. These stresses combine to cause intrauterine growth retardation, severe growth stagnation in infancy and early childhood [133;134] and weight loss in adults during this season. Highly significant month-of-birth effects could be shown with the highest mortality in births from July to December. At an early age, deaths were similar in groups born between January and June and during July and December, but from the age of 15 those born in the hunger season had a significantly greater mortality. After age 35, the risk of premature death for those born in the hunger season was 10.4 times higher compared to those not born in the hunger season. Of the 48 deaths occurring after the age of 15, 17 cases (35%) were infection-related. These results suggest that prenatal or early postnatal events affect the future health of rural Gambians in a manner first manifested around puberty and amplified with increasing age. The early *in utero* sensitivity of the immune system to malnutrition would fit with the observation that babies born up to two to three months after the peak of the hungry season remain vulnerable, suggesting that fetal growth impairment in mid-pregnancy may have a carry-over effect even when maternal nutrition improves in late pregnancy.

In animals, nutrient restrictions in pregnancy may have significant effects upon the immune function of the offspring [1]. Langley et al. [135] examined the effects of low protein diets fed to rats before conception and during pregnancy on the acute phase response initiated by an *Escherichia coli* endotoxin challenge in the adult offspring. The general trend was for the response to be attenuated by low protein diets. This study showed a complex, non-linear effect of dietary manipulation on the acute phase response. Rats exposed to the 9% and 12% casein diet (g casein/100 g diet; the minimum protein requirement for pregnant rats is reported to be 12 %), showed a more severe impairment of response than did those exposed to the 6% casein diet.
Beach et al. [136;137] fed mice a diet moderately deficient in zinc (5 ppm zinc versus 100 ppm zinc in the control diet) from day 7 of gestation until parturition. Offspring of these mice showed depressed immune function through 6 months of age. In addition, the second and third generations, all of which were fed the normal control diet, continued to manifest reduced immunocompetence, although not to the same degree as in the first generation. It is believed that the postnatal effects of zinc depletion during gestation were due to an effect in development rather than to the persistence of abnormal plasma zinc levels (as these were within normal ranges). The mechanism whereby zinc or other nutrients influence immune ontogeny in subsequent generations remains unclear.

# 6. Discussion

The intrauterine nutritional programming hypothesis has attracted increasing interest during the last decade. It has given rise to a series of epidemiological studies, which have shown associations between maternal nutrition and adult disease in offspring. In addition, a variety of experimental animal studies have given clues to mechanisms of programming. A series of review articles by a variety of research groups have been published [7,10,1,62,140] . The picture emerges of profound effects of maternal nutrition on morphologic and physiologic development *in utero*, which results in modulation of the risk of acquiring chronic diseases in adulthood. The observations that an association between intrauterine nutrition and adult disease risk can be detected although they occur some five decades in time apart gives an indication of the potential importance of such findings. On the other hand, many studies have shown the impact of adult lifestyle including nutrition on the risk of chronic diseases. It seems that adult lifestyle may modulate the risk of chronic diseases within the window of possible variation programmed *in utero*. If this hypothesis holds, the prevention of chronic diseases could be improved by optimising nutrition in pregnancy. With the current scarcity of pertinent data the extent of possible health benefits is hard to estimate. However, the diseases implicated in prenatal programming, such as cardiovascular disease, cancer, and diabetes have a major impact on public health. Therefore, the result of optimising nutrition in pregnancy in terms of prevention may be substantial, and warrants further study.

In epidemiologic studies, intrauterine nutrition has shown correlations with the risks in adult life of the most abundant chronic diseases currently present in the human population. Cancer, cardiovascular disease, obesity and diabetes, and infectious diseases have all shown associations with maternal nutrition. As most of these diseases have a high and increasing incidence in western societies, the study of their causes is of prominent public health importance. Epidemiologic studies in the area of intrauterine programming are inherently prone to bias and confounding in view of the time span between alleged cause and effect. Although most studies to date have been retrospectively, more recent studies have been set up prospectively (Matthews [75], Olsen [158], Lucas [556], Hornstra (personal communication)) , but it will take decades before their results in terms of chronic disease risk will emerge.

A variety of animal studies have addressed the physiologic basis for intrauterine programming. A general picture appears of the modulation of morphologic development of the conceptus by maternal nutrition. Morphologic effects have been reported in the hypothalamus-pituitary-adrenal system, the liver, the kidney, the pancreas and the mammae anlagen. These morphologic effects are reflected in physiological parameter settings, pertaining to e.g. hormonal physiology, metabolic enzyme activities, and stress and immune responsiveness. Animal studies clearly show that these settings may profoundly affect the risk of acquiring chronic diseases in adulthood.

Diverse nutritional factors have been employed for studying intrauterine programming in animal studies. The relevance of some of these nutritional interventions in view of current trends in human nutrition in the real world is not always clear. However, the most recent food consumption survey in the Netherlands (1998) [157] has shown changes in dietary fatty acid balance. Furthermore, a decrease in vegetables and fruit intake was observed, with a fibre intake that remains low as compared to the advised intake levels. These nutritional components have been implicated in intrauterine programming. In general terms of total caloric intake and macronutrient supply, the Dutch food consumption pattern is probably close to optimal [157] . However, balances between individual nutritional components may vary and pose risks for the developing embryo/fetus. Less favourable diets may occur especially in subgroups in society with a less favourable nutritional status, such as people with a low socio-economic status.

Further studies are needed to clarify the relationship between intrauterine nutrition and adult disease risk. As stated above, several longterm prospective epidemiologic studies have been initiated, which

will take years to deliver results. In the mean time, the study of mechanisms of programming should be continued. Animal studies have already given important insights into developmental consequences of variations in intrauterine nutrition. Such studies should be performed using nutritional interventions that mimick changes in nutrition occurring in society. Obvious nutritional candidates in this area are fatty acids and fibres, but other factors will undoubtedly appear in the future. This research will be instrumental in forming the basis for an adequate nutritional education of pregnant women, aimed at the prevention of chronic diseases in their offspring in later life.

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# *Appendix 1 Mailing list*

1. Dr. H.J. Schneider, Directeur-Generaal Volksgezondheid
2. Dr. W.H. van Eck, wnd. directeur Directie Gezondheidsbeleid
3. Prof. dr. J.J. Sixma, voorzitter Gezondheidsraad
4. Ir. R. Top, directie Gezondheidsbeleid, VWS
5. S.M.C. Potting, directie Gezondheidsbeleid, VWS
6. Drs. J. de Stoppelaar, directie Gezondheidsbeleid, VWS
7. Dr.ir. M.W.J. Wolfs, Hoofdinspecteur Food, IWV
8. Mr. ing. G.J.B. Koenen, Algemene Directie IWV
9. Ir. B.C. Breedveld, Voedingscentrum
10. Prof. dr. G. Hornstra, Universiteit Maastricht
11. Dr. R. van Houwelingen, Universiteit Maastricht
12. Drs. R.H.M. de Groot, Universiteit Maastricht
13. Drs. E.C. Bakker, Universiteit Maastricht
14. Drs. S. Otto, Universiteit Maastricht
15. Drs. P. Rump, Universiteit Maastricht
16. Dr. J.H. Brussaard (TNO-Voeding, Zeist)
17. Dr. A.H. Severs (TNO Vitamine Informatie Bureau, Zeist)
18. Depot Nederlandse Publicaties en Nederlandse Bibliografie
19. Directie RIVM
20. Prof. dr. ir. D. Kromhout (SB2)
21. Prof. dr. P.W.J. Peters (BIS)
22. Prof. dr. ir. J.C. Seidell (CZE)
23. Dr. ir. H.J.G.M. Derks (LGO)
24. Dr. P. van Zoonen (LOC)
25. Dr. ir. E. Lebret (LBM)
26. Dr. H.B. Bueno de Mesquita (CZE)
27. Ir. H. van Egmond (ARO)
28. Drs. A.G.A.C. Knaap (CSR)
29. Dr. A.K.D. Liem (LOC)
30. Dr.ir. M.C. Ocké (CZE)
31. Dr. ir. E.H.J.M. Jansen (LEO)
32. Dr. C.F. van Kreyl (LEO)
33. Ing. E. Schenk (LEO)
34. Drs. M.Q.I. Spanjersberg (LEO)
35. A. Verhoef (LEO)
36. Dr. W. Vleeming(LEO)

37. Prof. dr. D.J. de Wildt (LEO)
38. Prof. W. Slob (LEO)
39-41. Auteurs
42. Hoofd Bureau Voorlichting en Public Relations (RIVM)
43. Bureau Rapportenregistratie (RIVM)
44-58. Bureau Rapportenbeheer (RIVM)
59. Bibliotheek RIVM
60-64. Reserve-exemplaren