Maternal and fetal origins of cardiovascular disease

The Generation R Study

Acknowledgements

Maternal and Fetal Origins
of Cardiovascular Disease
The Generation R Study

Maternale en foetale origine van hart en vaatziekten

Het Generation R Onderzoek

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. S.W.J. Lamberts

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op
woensdag 24 mei 2006 om 13.45 uur

door

Vincent Wilfred Vishal - Kapoor Jaddoe
geboren te Zaandam.

Promotiecommissie

Promotoren:	Prof.dr. A. Hofman
	Prof.dr. H.A. Büller
Overige leden:	Prof. D.J.P. Barker
	Prof.dr. A.J. van der Heijden
	Prof.dr. E.A.P. Steegers
Copromotor:	Dr. J.C.M. Witteman

Pa, Grace, Raymond en Gillian

Contents

Manuscripts based on this thesis

Chapter 2
Jaddoe VWV, Witteman JCM. Hypotheses on the fetal origins of adult diseases: contributions of epidemiological studies. Eur J Epidemiol 2006;21:91-102.

Chapter 3.1
Hofman A, Jaddoe VWV, Mackenbach JP, Moll HA, Snijders RFM, Steegers EAP, Verhulst FC, Witteman JCM, Büller HA. Growth, development and health from early fetal life until young adulthood. The Generation R Study. Paediatr Perinat Epidemiol 2004;18:61-72.

Chapter 3.2
Jaddoe VWV, Mackenbach JP, Moll HA, Steegers EAP, Tiemeier H, Verhulst FC, Witteman JCM, Hofman A. The Generation R Study: design and cohort profile. Eur J Epidemiol 2006; in press.

Chapter 4.1
Jaddoe VWV, Troe JWM, Hofman A, Mackenbach JP, Moll HA, Steegers EAP, Witteman JCM. Active and passive maternal smoking patterns in pregnancy and the risk of low birth weight and preterm birth. The Generation R Study. Submitted

Chapter 4.2
Jaddoe VWV, Verburg BO, de Ridder MAJ, Hofman A, Mackenbach JP, Moll HA, Steegers EAP, Witteman JCM. Maternal smoking and fetal growth characteristics in different periods of pregnancy. The Generation R Study. Submitted

Chapter 4.3
Jaddoe VWV, Bakker R, Hofman A, Mackenbach JP, Moll HA, Steegers EAP, Witteman JCM. Maternal alcohol consumption in pregnancy and the risk of low birth weight and preterm birth. The Generation R Study. Submitted

Chapter 5.1
Jaddoe VWV, van Osch - Gevers M, Geelhoed JJ, Verburg BO, Hofman A, Moll HA, Steegers EAP, Helbing WA, Witteman JCM. Fetal growth characteristics and left cardiac structure in infancy. The Generation R Study. Submitted

Chapter 5.2
Jaddoe VWV, de Ridder MAJ, van den Elzen APM, Hofman A, Uiterwaal CSPM, Witteman JCM. Maternal smoking in pregnancy and blood pressure development in the offspring. A 27-year follow-up study. Submitted

Chapter 5.3
Jaddoe VWV, van den Elzen APM, Hofman A, Uiterwaal CSPM, Witteman JCM. Maternal smoking in pregnancy and cholesterol development in the offspring. A 27-year follow-up study. Submitted

Chapter 5.4
Jaddoe VWV, Morks AN, van den Elzen APM, Hofman A, Witteman JCM. Early life characteristics and arterial stiffness in adulthood. Submitted

Introduction

In the past two decades, epidemiological studies have demonstrated associations of low birth weight with cardiovascular disease and its risk factors. The fetal origins hypothesis proposes that an adverse fetal environment leads to developmental adaptations that permanently program the fetus' structure, physiology and metabolism [1]. This programming is in favor of short-term survival and leads to fetal growth retardation and low birth weight. Long-term effects of this programming would be detrimental and lead to several health problems in adulthood including cardiovascular disease. Based on this hypothesis, the search for the origins of cardiovascular disease has recently been extended from epidemiological studies in adults and children to new studies focused on fetal and early postnatal life.

The fetal origins hypothesis was the main point of departure for studies presented in this thesis. Unravelling the mechanisms underlying the associations of low birth weight with cardiovascular disease may eventually lead to new strategies for identification of groups at risk and prevention. The general aim of the studies presented in this thesis was to identify pathways leading from adverse fetal exposures to suboptimal fetal growth patterns and subsequently to risk factors for cardiovascular disease in later life. The adverse fetal exposure of main interest was maternal smoking in pregnancy, which is the single most important determinant of low birth weight in Western countries.

In *chapter 2*, a descriptive review is presented focused on epidemiological studies testing the various hypotheses proposed to explain the associations of low birth weight with cardiovascular disease in later life. In *chapter 3* the Generation R Study is presented. This is a recently started cohort study designed to study growth, development and health from fetal life until young adulthood. Data from the Generation R Study were used for studies presented in *chapter 4*. Studies in this chapter deal with the effect of maternal smoking and alcohol consumption in pregnancy on fetal growth. Studies in *chapter 5* deal with the postnatal cardiovascular consequences of fetal growth retardation and maternal smoking in pregnancy. The long-term effect of maternal smoking was studied in the Epidemiological Prevention Organization Zoetermeer (EPOZ) Study, which comprises children initially aged 5 to 19 years and currently followed for 27 years. Finally, in *chapter 6,* the results described in this thesis are placed in a broader context, some methodological aspects are discussed and suggestions for further research regarding this topic are presented.

Reference

1.   Barker DJ. Fetal origins of coronary heart disease. BMJ 1995;311:171-4.

Hypotheses on the fetal origins of adult diseases

2

Abstract

Epidemiological studies have demonstrated associations of low birth weight with cardiovascular disease, type 2 diabetes and their risk factors in adult life. These findings led to sharp debates in the literature concerning potential methodological study flaws and the effect size and causality of the associations. More recent studies seem to have overcome most methodological flaws and suggest a small effect size of low birth weight on adult diseases for the individual. However, the effect size may still be important on a population level. Various causal pathways have been hypothesized as mechanisms underlying these associations. These hypotheses have proposed central roles for 1) fetal undernutrition; 2) increased cortisol exposure; 3) genetic susceptibility; and 4) accelerated postnatal growth. These hypotheses have been studied in various epidemiological study designs. Thus far, it is still not known which mechanisms underlie the associations between low birth weight and diseases in adult life. The causal pathways seem to be complex and may include combined environmental and genetic mechanisms in various periods of life. Well-designed epidemiological studies are necessary to estimate the population effect size and to identify the underlying mechanisms. This knowledge is needed to develop strategies for identifying groups at risk and prevention focused on early life.

In the past 15 years, many studies demonstrated associations between low birth weight and cardiovascular disease, type 2 diabetes and their risk factors in adult life. These findings led to sharp debates concerning possible explanations. Researchers seem to be divided into almost religious camps of non-believers and believers [1]. Non-believers consider methodological study flaws as explanations for the associations. Believers consider the associations as causal and discuss possible biological underlying mechanisms (Table 1). This review is focused on published epidemiological studies designed to identify the mechanisms underlying the associations between low birth weight and adult diseases.

Table 1. Subjects most debated

Inconsistencies
Incomplete statistical analysis
(Residual) confounding
Selection bias
Causality
Strength and public health impact
Underlying mechanisms

Methodological flaws

The first studies demonstrating associations between low birth weight and diseases in later life led to methodological concerns including confounding, inconsistent results and selection bias [2, 3]. These studies were based on retrospective data from birth-cohorts and not able to adjust for potential confounders. More recent studies were able to take socio-economic status, life style factors and family history of cardiovascular disease into account in large cohorts and still demonstrated the associations [4-6]. Residual confounding could still be the case due to insufficiently measured variables including parental or current offspring socio-economic status, parental smoking habits and cardiovascular disease and gestational age. Exploration and careful interpretation of the types and extent of confounding in the associations is needed. Even when the confounding effects of these variables will be quantified, it could be difficult to interpret how they affect physiological mechanisms underlying the epidemiological associations. For example, in case of a true causal pathway from a suboptimal maternal diet to an impaired fetal growth and diseases in later life, taking socio-economic status into account might lead to disappearance of the association if socio-economic status is related to maternal diet. Biological knowledge and pre-specified hypotheses are necessary for careful interpretation of these complex associations.

Other objectives from non-believers are the inconsistencies in growth characteristics in early life used in the various studies. Because birth weight is only a proxy for fetal

growth and development, major efforts have been made to find historical data sets with more detailed growth characteristics at birth. This has led to many studies showing associations of weight, head circumference, abdominal circumference and ponderal index (kg/m^3) at birth, placental weight and weight at 1 year with cardiovascular disease, type 2 diabetes and their risk factors in adult life [7, 8]. Although specific explanations have been proposed for almost all of these separate measures at birth and their associations with diseases in later life, the results have rarely been replicated. Weight is the only birth characteristic consistently associated with cardiovascular disease and type 2 diabetes in adult life [9, 10]. Length, head circumference and abdominal circumference are more difficult to measure at birth than weight. The number of studies with these specific measurements is smaller and random error may be larger. Therefore, of all anthropometric measures at birth, weight may be most likely to demonstrate significant associations with any outcomes in adult life.

Not only the use of different growth characteristics at birth but also the effect of weight in later life on the associations is subject of debate. Most studies examining the associations of birth weight with adult diseases simply adjust for current weight in their statistical models. However, in this case, the effect estimates of low birth weight on for example blood pressure in adult life, depend on the separate associations between birth weight and blood pressure, between birth weight and current weight and between current weight and blood pressure [11]. If the associations between birth weight and adult diseases are absent or weak, adjusting for current weight frequently reveals stronger and negative associations. The interpretation of this effect could be that if subjects grow to the same weight in adulthood, the ones with lower birth weight have an increased blood pressure. Lucas et al. proposed that in this case, not birth weight itself, but postnatal change from birth weight to current weight may be associated with an increased blood pressure [12]. They proposed four regression models to analyze separate effects of birth weight, postnatal weight change and current weight.

Criticisms frequently have pointed to the enormous loss at follow-up in the historical cohort studies. In the initial studies, results were based on only 5 to10% of the original birth-cohorts [2]. Although selection bias would only arise if the associations between low birth weight and diseases in later life are different between those studied and those lost to follow-up, distortion of the observed associations is more likely to occur in case of massive loss to follow-up. More recent studies had follow-up rates of more than 90% and still demonstrated associations between birth weight and diseases in later life [4-6].

Much of the current skepticism is not focused on the methodological flaws anymore, but on the strength, public health impact and causality of the associations [13]. A meta-analysis of studies reporting associations between birth weight and blood pressure in later life demonstrated an increase of systolic blood pressure of 0.4 mmHg per kilogram decrease in birth weight [9]. This is much lower than previously suggested esti-

mates (increase of 2 to 4 mmHg per kilogram decrease in birth weight). A similar small effect was demonstrated in a meta-analysis of studies reporting associations between low birth weight and cholesterol in adult life [14]. Publication bias and inappropriate adjustment for potential confounders were suggested as explanations for the earlier suggested stronger estimates [15]. Although the real effect sizes may be small for the individual, they could still be causal and important on a population level. Evidence for the causality of the associations comes from experimental animal studies that have been reviewed previously [16]. Causality is also suggested by presence of the associations between low birth weight and diseases in adult life in epidemiological studies with minimal loss to follow-up and appropriate adjustment for potential confounders in early and adult life [4-6].

Thus far, not all of the mentioned methodological issues have been clarified yet. However, the concept that children born with a low birth weight have an increased risk of developing cardiovascular disease and type 2 diabetes has gradually been recognized as potentially scientifically plausible.

Underlying causal mechanisms

Various causal pathways underlying the associations between low birth weight and adult diseases have been hypothesized. These hypotheses proposed central roles for respectively 1) fetal undernutrition; 2) increased cortisol exposure; 3) genetic susceptibility; and 4) accelerated postnatal growth [17-20] (Table 2). These hypotheses are supported

Table 2. Hypotheses on the fetal origins of adult diseases

Fetal undernutrition [17, 21]

Suboptimal fetal nutrition leads to developmental adaptations that permanently program the fetus' structure, physiology and metabolism. This programming is in favor of short-term survival and leads to low birth weight. Long-term effects of this programming are detrimental and lead to cardiovascular disease and type 2 diabetes. This hypothesis has gradually modified into a more general developmental plasticity model in which various fetal and postnatal factors could lead to programming responses.

Steroids exposure and the hypothalamic-pituitary-adrenal axis [18]

Increased exposure to cortisol leads to both low birth weight and diseases in later life. Impaired placental 11-beta-hydroxysteroid dehydrogenase enzyme activity, which converts cortisol into inactive cortisone, leads to increased fetal exposure to cortisol, low birth weight and developmental adaptations in cardiovascular and metabolic systems. These adaptations subsequently predispose the individual to cardiovascular disease and type 2 diabetes.

Genetic susceptibility [19]

Genetic variations related to insulin resistance, impaired insulin secretion or cardiovascular development lead prenatally to impaired fetal growth and postnatally to type 2 diabetes and cardiovascular disease.

Accelerated postnatal growth [20]

Rapid postnatal growth instead of birth weight per se leads to diseases in later life. Rapid childhood growth occurs in most children with low birth weight and could lead to metabolic and vascular changes and subsequently to diseases in later life.

by small studies in humans and studies in animals. Since findings from these studies cannot easily be extrapolated to the general population, we reviewed epidemiological studies focused on these hypotheses.

Fetal undernutrition

The first hypothesis explaining the associations between birth weight and diseases in later life was formulated by Barker and his colleagues from Southampton and is focused on fetal undernutrition and programming [17, 21]. Suboptimal fetal nutrition would lead to developmental adaptations that permanently program the fetus' structure, physiology and metabolism. This programming would be in favor of short-term survival and lead to low birth weight. Long-term effects of this programming would be detrimental and lead to cardiovascular disease and type 2 diabetes. Based on more recent animal and human studies, this hypothesis has gradually been modified into a general developmental plasticity model in which various fetal and postnatal environmental factors lead to programming responses [22]. Low birth weight is in this hypothesis a proxy for suboptimal fetal growth and development and not the causal factor per se. Measures of an adverse fetal nutrition may be stronger risk factors for adult diseases than birth weight. The fetal nutrition supply line includes maternal anthropometrics and diet, placenta function and the fetus' own metabolism to use nutrients. This hypothesis has been tested in epidemiological studies by relating various determinants of an adverse fetal nutrition to diseases and their risk factors in adult life.

Studies that examined the direct effect of maternal anthropometrics on diseases in the offspring demonstrate conflicting results [23-30] (Table 3). Low maternal body mass index in early and late pregnancy was not associated with high blood pressure but was associated with early measures of insulin resistance in the offspring [24-29]. These results were adjusted for birth weight. The effect of maternal weight gain in pregnancy on development of cardiovascular disease and type 2 diabetes in the offspring is not known. Maternal triceps skin fold thickness in pregnancy was inversely associated with blood pressure in the offspring [27, 29, 30]. From all maternal anthropometric measures, maternal skin fold thickness seems to be the most consistent independent determinant of blood pressure in the offspring. It has been suggested that skin fold thickness may be a more stable measure of maternal nutritional status than body mass index in early or late pregnancy [27].

The effect of maternal diet on the development of risk factors for cardiovascular disease and type 2 diabetes in the offspring has been studied in retrospective cohort studies focused on total energy and macronutrient and micronutrient intake. Follow-up studies in subjects who have been exposed prenatally to the famine during World War II have been conducted in cohorts in Amsterdam in the Netherlands and in Leningrad in Russia. These historical disasters provided the opportunity to study long-term effects

Table 3. Determinants of fetal nutrition studied in association with risk factors for cardiovascular disease and type 2 diabetes

First author (year)	Main finding
Maternal anthropometrics: weight and body mass index	
Forsen (1997) [23]	Obesity in short stature mothers is associated with death from coronary heart disease in the offspring.
Shiell (2000), Mi (2000) [24, 25]	Low maternal body mass in early and late pregnancy is associated with early measures of insulin resistance, but not with blood pressure in the adult offspring. The effect is independent of their offspring birth and current weight.
Margetts (1991), Godfrey (1994), Laor (1997), Clark (1998) [26-29]	Maternal weight in pregnancy is not associated with their offspring's blood pressure in childhood and adolescence.
Maternal anthropometrics: triceps skinfold thickness	
Godfrey (1994), Clark (1998), Adair (2001) [27, 29, 30]	Maternal triceps skin fold thickness in pregnancy is inversely associated with blood pressure in childhood in their offspring in childhood.
Maternal diet: energy intake	
Roseboom (2000), Ravelli (1998), Stanner (1997), Sparen (2004) [31-34]	Maternal exposure to the Dutch famine during pregnancy is associated with impaired glucose tolerance and coronary heart disease in the adult offspring. These findings could not fully be replicated in the Leningrad siege study.
Maternal diet: macronutrients	
Campbell (1996), Shiell (2001) [35, 36]	Maternal protein and carbohydrate intake are associated with high blood pressure in the offspring.
Shiell (2000) [24]	Increased maternal intake of fat and protein is associated with reduced insulin secretion in the adult offspring suggesting pancreatic dysfunction. No associations were found with measures of insulin resistance.
Maternal diet: micronutrients	
Belizan (1997), Hatton (2003), Gillman (2004), Morley (2004) [38-41]	Maternal calcium intake during pregnancy is associated with lower blood pressure in the infant offspring. Findings in these studies were independent from birth weight.
Placenta: weight	
Godfrey (2000) [42]	Placenta weight is not consistently associated with risk factors for cardiovascular disease and type 2 diabetes in adulthood.
Placenta: haemodynamic function	
Smith (2001) [43]	Placenta dysfunction, like pre-eclampsia, is associated with low birth weight and high blood pressure in the offspring.

of extreme maternal undernutrition in pregnancy. Studies in the Amsterdam cohort demonstrated that prenatal exposure to the famine is associated with coronary heart disease and impaired glucose tolerance in adult life [31, 32]. These associations have been replicated only partly in the Leningrad cohort [33, 34]. More detailed information about the effect of macronutrient and micronutrient intake has come from follow-up

studies in offspring whose mothers have participated in nutritional studies during pregnancy. Observational studies demonstrated complex interactions between maternal protein and carbohydrate intake as determinants of blood pressure and insulin secretion in adults but not in the infant offspring [24, 35-37]. These findings have not been replicated in other studies. The only micronutrient intake during pregnancy studied in relation to risk factors for cardiovascular disease in the offspring is calcium intake. Since calcium supplementation in pregnancy might reduce pregnancy-induced hypertension and pre-eclampsia, various studies of calcium supplementation in pregnancy have been conducted. These studies provided the opportunity to study the effect of maternal calcium supplementation on the offspring's blood pressure. Four prospective studies including two randomized controlled trials, demonstrated that maternal calcium intake during pregnancy is associated with lower blood pressure in infant offspring [38-41]. These findings were independent from birth weight.

Placenta function is probably the most important determinant of fetal nutrition in Western countries. Proxies of placental function studied in relation to adult diseases in the offspring in epidemiological studies include placental weight and haemodynamic function. The associations between placental weight or placental/birth weight ratio and cardiovascular disease and type 2 diabetes have been reviewed previously [42]. No consistent associations were found. Pre-eclampsia, a more severe form of haemodynamic placental dysfunction, is associated with both later cardiovascular disease in the mother and high blood pressure in the offspring [43]. The less severe form of placental dysfunction, measured as sub-clinical blood flow alterations in the uterine artery, is associated with low birth weight but the association of this form of placental dysfunction with cardiovascular disease and type 2 diabetes in the offspring has not been studied yet.

The last step in the fetal supply line, the fetus' own metabolism to use nutrients, is not easy to study in epidemiological studies. The fetal undernutrition hypothesis suggests fetal programming in various organs. Programming effects that have been proposed include an impaired nephron number and increased arterial stiffness [44, 45]. Impaired nephron number or increased arterial stiffness could be intermediates in the associations of fetal nutrition or low birth weight with hypertension in adult life. Autopsy studies demonstrated that the nephron number is positively associated with birth weight and inversely associated with blood pressure [46, 47]. Renal volume measured by ultrasound has been suggested to be a proxy for nephron number suitable for use in epidemiological studies and is positively associated with birth weight and negatively associated with blood pressure [48, 49]. The intermediate role of nephron number or renal volume in the association between low birth weight and hypertension in adult life has not yet been studied directly. Arterial stiffness, a strong determinant of blood pressure, seems to be negatively associated with birth weight in children and adults [50, 51].

It is not known whether increased arterial stiffness is an intermediate in the association between low birth weight and hypertension in later life.

Thus far, the fetal undernutrition hypothesis is not strongly supported by epidemiological studies. Results of epidemiological studies using measures of fetal nutrition are inconsistent and can not explain the associations between low birth weight and cardiovascular disease or type 2 diabetes in adult life. The most robust finding in these studies seems to be the association between maternal calcium supplementation and lower blood pressure in the offspring. The intermediate role of proposed programming mechanisms including impaired nephron number or increased arterial stiffness cannot directly be concluded from current epidemiological studies.

Steroids exposure and the hypothalamic-pituitary-adrenal axis

Edwards et al. hypothesized that an increased cortisol exposure leads to both low birth weight and diseases in later life [18]. Placental 11-beta-hydroxysteroid dehydrogenase enzyme (11-βHSD) converts maternal cortisol into inactive cortisone. They suggested that impaired activity of the placental 11-βHSD would lead to increased fetal exposure to cortisol and subsequently to fetal growth retardation and developmental adaptations in cardiovascular and metabolic systems. These adaptations would subsequently predispose the individual to cardiovascular disease and type 2 diabetes in later life. Increased fetal exposure to cortisol may be the result of both absolute and relative impaired 11-βHSD due to increased maternal cortisol levels. This hypothesis is supported by experimental studies in animals, which have been reviewed in detail previously [52]. Environmentally or genetically determined common variations in 11-βHSD activity may explain the inverse association between birth weight and hypertension in the general population. However, no such variations have been reported yet. Epidemiological studies in humans are scarce mainly because of the difficulty of measuring 11-βHSD activity. Small studies have demonstrated that placental 11-βHSD gene expression is impaired in pre-eclampsia [53]. The enzyme activity is not associated with birth weight but seems to be associated with essential hypertension [54, 55]. The direct effect of placental 11-βHSD activity on adult blood pressure has not been studied in humans.

Increased cortisol levels may not only be the result of absolute or relative impaired 11-βHSD activity but also of other developmental changes in the hypothalamic-pituitary-adrenal axis [56]. These developmental changes may be the result of various environmental or genetic causes. Several measures of increased hypothalamic-pituitary-adrenal axis activity are associated with low birth weight [57, 58]. Instead of causing both low birth weight and cardiovascular disease and type 2 diabetes in later life, increased cortisol levels may also be an intermediate in the causal pathway linking low birth weight with diseases in later life. From current epidemiological studies, it is not clear whether

increased cortisol exposure is just one of the results of developmental changes in early life or an intermediate in the causal pathway.

Genetic susceptibility

Hattersley and Tooke proposed the fetal insulin hypothesis [19]. They hypothesized that genetic variations related to insulin resistance lead prenatally to impaired insulin mediated growth and postnatally to type 2 diabetes and cardiovascular disease. This hypothesis is primarily based on studies demonstrating rare monogenetic variants leading to both low birth weight and impaired insulin secretion or insulin sensitivity. More common genetic variants related to insulin metabolism or cardiovascular development may be an explanation for the association between low birth weight and diseases in later life in the general population. Epidemiological study designs used to examine this hypothesis include follow-up studies of parents from children with low birth weight, twin studies and genetic association studies.

Mothers with pregnancies complicated by preterm delivery, fetal growth restriction or pre-eclampsia have an increased risk of developing ischemic heart disease, cerebrovascular accidents and insulin resistance many decades later [43, 59, 60]. The associations of low birth weight in the offspring with later disease in the mothers themselves are stronger than the associations of low birth weight with diseases in adult life in the offspring. Pre-eclampsia and cardiovascular disease in women may have the same genetic and environmental determinants. Similar follow-up studies have been conducted in fathers from children with low birth weight. Low birth weight in the offspring is associated with development of type 2 diabetes in fathers in Western populations [61-65]. These findings suggest that genetic variants lead to both low birth weight and type 2 diabetes.

Twin studies seem to provide an optimal model to distinguish between environmental and genetic variants as underlying mechanisms. Differences in birth weights within monozygotic twin pairs are more likely to be the result of unequal nutrients supply. Because of their similar genetic background, associations of low birth weight with diseases in later life within only monozygotic twin pairs suggest environmental instead of genetic underlying mechanisms. Previous studies demonstrated associations in both monozygotic and dizygotic twin pairs, only in dizygotic twin pairs, and in neither monozygotic nor dizygotic twin pairs [66-77]. Thus although promising, twin studies have been inconclusive and failed to give insight in the mechanisms underlying the associations between low birth weight and diseases in adult life [78].

Genetic association studies could reveal common genetic variants explaining the associations between low birth weight and diseases in later life. Recent studies suggested that various common genetic variants previously linked to type 2 diabetes or cardiovascular disease are associated with low birth weight. Genetic variants studied

Table 4. Common genetic variants studied to explain the associations between low birth weight and diseases in later life

First author (year)	Main finding
Insulin gene variable number of tandem repeat (INS VNTR)	
Dunger (1999) [80]	III/III genotype is associated with increased head circumference, length and weight at birth.
Ong (1999) [81]	III/III genotype is associated with increased risk of developing type 2 diabetes in adults without postnatal growth realignment but not with birth weight.
Bazaes (2003) [82]	III/III genotype is associated with increased fasting insulin and insulin secretion in infants.
Ong (2004) [83]	III/III genotype is associated with larger head circumference at birth. Genotype interacts with early postnatal weight gain in determining childhood body mass index, weight and waist circumference.
Benett (2004), Mitchell (2004) [84, 85]	III/III genotype is not associated with size at birth in children with and without postnatal growth realignment.
Insulin like growth factor-1 gene (IGF-1)	
Vaessen (2001) [86]	Absence of 192-bp allele is associated with impaired height, increased risk of type 2 diabetes and myocardial infarction and lower IGF-1 levels.
Vaessen (2002) [87]	Absence of 192-bp allele is associated with low birth weight and postnatal weight gain.
Frayling (2002) [88]	Absence of 192-bp allele is weakly associated with increased risk of type 2 diabetes and higher IGF-1 levels.
Arends (2002) [89]	Carrying the 191 allele is associated with persistent smaller head circumference and lower IGF-1 levels.
Peroxisome proliferator-activated receptor gamma2 gene (PPAR-γ2)	
Deeb (1998) [95]	Pro12Pro genotype is associated with higher body mass index, impaired insulin sensitivity and type 2 diabetes.
Eriksson (2002) [91]	Pro12Pro genotype modifies the association between low birth weight and insulin resistance.
Yliharsila (2004) [92]	Pro12Pro genotype modifies the association between low birth weight and hypertension.

most frequently in different cohorts include the insulin variable number tandem repeat (INS VNTR) gene variant, various variants in the insulin like growth factor-1 (IGF-1) gene and variants in the peroxisome proliferator-activated receptor gamma 2 (PPAR-γ2) gene [79-92] (Table 4). The biological roles of these gene variants were demonstrated in studies relating them with the gene expression of insulin and insulin like growth factor-2 (IGF-2), with IGF-1 serum levels and with insulin metabolism, respectively [86, 93-95]. To date, these gene variants seem to explain part of the associations between low birth weight and diseases in later life. However, as frequently seen in genetic association studies, current studies did demonstrate conflicting results [96].

Accelerated postnatal growth

Singhal and Lucas proposed the growth acceleration hypothesis suggesting that primarily rapid postnatal growth instead of low birth weight per se leads to diseases in later life [20]. Rapid postnatal growth rate occurs in most children with low birth weight who demonstrate a catch-up growth in infancy in the first 6 to 24 months [97]. The associations between low birth weight and diseases in later life may be based on this compensatory accelerated postnatal growth. Studying this hypothesis and the separate influences of birth weight and postnatal growth on development of diseases in later life requires detailed growth data with a long-term follow-up. The association between low birth weight and cardiovascular disease and type 2 diabetes is amplified by a high body mass index in childhood and adult life [4, 98, 99]. Even small body mass index percentile changes without marked childhood obesity in low birth weight children leads to an increased risk of impaired insulin resistance in adult life [100]. Prospective studies demonstrated that weight gain in the first two years and early adiposity rebound are, independent of birth weight, associated with development of childhood obesity [101, 102]. Increased early postnatal weight gain induced by nutrient enriched formula feeding is associated with increased blood pressure, adverse lipid profile and insulin resistance in later life [103, 104]. These studies support the growth acceleration hypothesis. The difficulty of studying this hypothesis is that fetal and postnatal growth are not independent and complex models are required to analyze separate effects of different growth periods [12]. The association between early weight gain and diseases in later life may not be causal per se and could be explained by both environmental and genetic mechanisms.

New epidemiological studies

Recent epidemiological studies examining the associations of low birth weight with cardiovascular disease and type 2 diabetes in adult life have overcome some but not all methodological issues. Further exploration of the role of potential confounders and of the population effect size is necessary. Although animal and epidemiological studies support the proposed hypotheses, none of these current hypotheses fully explain the associations. The causal pathways linking low birth weight to cardiovascular disease and type 2 diabetes in later life may be complex and probably include combined several environmental and genetic mechanisms in various periods of life. Parts of these causal pathways can only be studied in experimental animal or small studies in humans. New well-designed epidemiological studies are necessary to overcome the existing methodological issues, to quantify the population effect size and to identify the combined
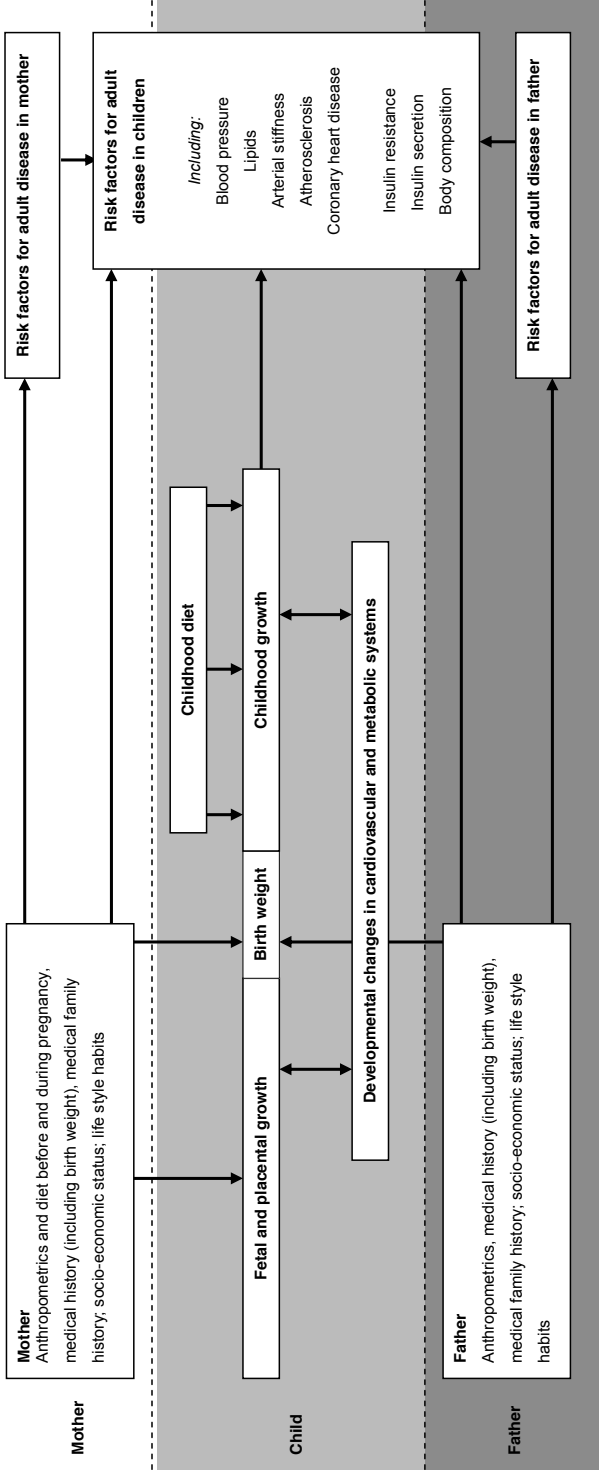
causal pathways underlying the associations. These studies should combine the following approaches:

1) An optimal epidemiological approach to overcome earlier methodological flaws and biases and to establish the population effect size;
2) A life course approach to identify fetal and postnatal growth and developmental patterns and their determinants associated with cardiovascular disease or type 2 diabetes in adult life;
3) A combined approach into possible environmental and genetic variations underlying the associations.

The epidemiological study design that seems most suitable for these approaches is a population-based prospective cohort study beginning in pregnant or even pre-pregnant women and their partners in which the offspring is followed from fetal life into adulthood (Figure 1). This design is ideal for data quality, taking account of potential confounders and identifying growth patterns at risk. Studies with this design have been started worldwide recently and will soon start to publish results [105, 106]. Although surrogate outcomes in childhood are available since risk factors for cardiovascular disease and type 2 diabetes track to some extent from childhood to adulthood, the major limitation of this design is the long period needed for the adult diseases to develop. This limitation can be overcome by using existing retrospective follow-up studies or register-based studies with longitudinal data on at least childhood growth and development of risk factors of cardiovascular disease and type 2 diabetes. The main advantage of this design is the earlier availability of outcomes directly related to cardiovascular disease and type 2 diabetes. This design may not be able to take account of all possible confounders and is not suitable to study the effect of fetal or early postnatal influences.

Studies with these approaches should be able to overcome the methodological issues and identify mechanisms in the causal pathways that underlie the associations. Maybe more importantly, they should lead to a rational debate and discussion between the non-believers and believers and close the gap between them. After more than 15 years of studies into the fetal origins of adult diseases, it is time to clarify the importance, effect size and causality of the associations and, if indeed causal, to develop strategies for identifying groups at risk and prevention focused on the earliest phase of life.

Figure 1. Models for studying the hypotheses on fetal origins of adult diseases in epidemiological studies

This model demonstrates core associations that have to be studied to clarify the mechanisms underlying the associations between low birth weight and diseases in later life. The mid-area demonstrates associations in children that have to identify growth patterns and developmental changes in fetal life and early childhood that lead to risk factors of diseases in adulthood. The upper and lower areas demonstrate associations in mother and father, respectively, that could give insight in both determinants of prenatal and postnatal growth patterns and in the underlying environmental and genetic mechanisms.

References

1. Poulter NR. Birthweights, maternal cardiovascular events, and Barker hypothesis. Lancet 2001;357:1990-1.
2. Kramer MS, Joseph KS. Enigma of fetal/infant-origins hypothesis. Lancet 1996;348:1254-5.
3. Paneth N, Susser M. Early origin of coronary heart disease (the "Barker hypothesis"). BMJ 1995;310:411-2.
4. Frankel S, Elwood P, Sweetnam P, Yarnell J, Smith GD. Birthweight, body-mass index in middle age, and incident coronary heart disease. Lancet 1996;348:1478-80.
5. Curhan GC, Willett WC, Rimm EB, Spiegelman D, Ascherio AL, Stampfer MJ. Birth weight and adult hypertension, diabetes mellitus, and obesity in US men. Circulation 1996;94:3246-50.
6. Rich-Edwards JW, Colditz GA, Stampfer MJ, Willett WC, Gillman MW, Hennekens CH, et al. Birthweight and the risk for type 2 diabetes mellitus in adult women. Ann Intern Med 1999;130:278-84.
7. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. Lancet 1989;2:577-80.
8. Barker DJ, Godfrey KM, Osmond C, Bull A. The relation of fetal length, ponderal index and head circumference to blood pressure and the risk of hypertension in adult life. Paediatr Perinat Epidemiol 1992;6:35-44.
9. Huxley R, Neil A, Collins R. Unravelling the fetal origins hypothesis: is there really an inverse association between birthweight and subsequent blood pressure? Lancet 2002;360:659-65.
10. Newsome CA, Shiell AW, Fall CH, Phillips DI, Shier R, Law CM. Is birth weight related to later glucose and insulin metabolism?: A systematic review. Diabet Med 2003;20:339-48.
11. Tu YK, West R, Ellison GT, Gilthorpe MS. Why evidence for the fetal origins of adult disease might be a statistical artifact: The "reversal paradox" for the relation between birth weight and blood pressure in later life. Am J Epidemiol 2005;161:27-32.
12. Lucas A, Fewtrell MS, Cole TJ. Fetal origins of adult disease-the hypothesis revisited. BMJ 1999;319:245-9.
13. Kramer MS. Invited commentary: association between restricted fetal growth and adult chronic disease: is it causal? Is it important? Am J Epidemiol 2000;152:605-8.
14. Huxley R, Owen CG, Whincup PH, Cook DG, Colman S, Collins R. Birth weight and subsequent cholesterol levels: exploration of the "fetal origins" hypothesis. JAMA 2004;292:2755-64.
15. Schluchter MD. Publication bias and heterogeneity in the relationship between systolic blood pressure, birth weight, and catch-up growth: a meta analysis. J Hypertens 2003;21:273-9.
16. Gluckman PD, Hanson MA. Living with the past: evolution, development, and patterns of disease. Science 2004;305:1733-6.
17. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. Lancet 1993;341:938-41.
18. Edwards CR, Benediktsson R, Lindsay RS, Seckl JR. Dysfunction of placental glucocorticoid barrier: link between fetal environment and adult hypertension? Lancet 1993;341:355-7.
19. Hattersley AT, Tooke JE. The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. Lancet 1999;353:1789-92.
20. Singhal A, Lucas A. Early origins of cardiovascular disease: is there a unifying hypothesis? Lancet 2004;363:1642-5.
21. Barker DJ. Fetal origins of coronary heart disease. BMJ 1995;311:171-4.
22. Bateson P, Barker D, Clutton-Brock T, Deb D, D'Udine B, Foley RA, et al. Developmental plasticity and human health. Nature 2004;430:419-21.
23. Forsen T, Eriksson JG, Tuomilehto J, Teramo K, Osmond C, Barker DJ. Mother's weight in pregnancy and coronary heart disease in a cohort of Finnish men: follow up study. BMJ 1997;315:837-40.
24. Shiell AW, Campbell DM, Hall MH, Barker DJ. Diet in late pregnancy and glucose-insulin metabolism of the offspring 40 years later. BJOG 2000;107:890-5.

25. Mi J, Law C, Zhang KL, Osmond C, Stein C, Barker D. Effects of infant birthweight and maternal body mass index in pregnancy on components of the insulin resistance syndrome in China. Ann Intern Med 2000;132:253-60.

26. Margetts BM, Rowland MG, Foord FA, Cruddas AM, Cole TJ, Barker DJ. The relation of maternal weight to the blood pressures of Gambian children. Int J Epidemiol 1991;20:938-43.

27. Godfrey KM, Forrester T, Barker DJ, Jackson AA, Landman JP, Hall JS, et al. Maternal nutritional status in pregnancy and blood pressure in childhood. BJOG 1994;101:398-403.

28. Laor A, Stevenson DK, Shemer J, Gale R, Seidman DS. Size at birth, maternal nutritional status in pregnancy, and blood pressure at age 17: population based analysis. BMJ 1997;315:449-53.

29. Clark PM, Atton C, Law CM, Shiell A, Godfrey K, Barker DJ. Weight gain in pregnancy, triceps skinfold thickness, and blood pressure in offspring. Obstet Gynecol 1998;91:103-7.

30. Adair LS, Kuzawa CW, Borja J. Maternal energy stores and diet composition during pregnancy program adolescent blood pressure. Circulation 2001;104:1034-9.

31. Roseboom TJ, van der Meulen JH, Osmond C, Barker DJ, Ravelli AC, Schroeder-Tanka JM, et al. Coronary heart disease after prenatal exposure to the Dutch famine, 1944-45. Heart 2000;84:595-8.

32. Ravelli AC, van der Meulen JH, Michels RP, Osmond C, Barker DJ, Hales CN et al. Glucose tolerance in adults after prenatal exposure to famine. Lancet 1998;351:173-7.

33. Stanner SA, Bulmer K, Andres C, Lantseva OE, Borodina V, Poteen VV, et al. Does malnutrition in utero determine diabetes and coronary heart disease in adulthood? Results from the Leningrad siege study, a cross sectional study. BMJ 1997;315:1342-8.

34. Sparen P, Vagero D, Shestov DB, Plavinskaja S, Parfenova N, Hoptiar V, et al. Long term mortality after severe starvation during the siege of Leningrad: prospective cohort study. BMJ 2004;328:11.

35. Campbell DM, Hall MH, Barker DJ, Cross J, Shiell AW, Godfrey KM. Diet in pregnancy and the offspring's blood pressure 40 years later. BJOG 1996;103:273-80.

36. Shiell AW, Campbell-Brown M, Haselden S, Robinson S, Godfrey KM, Barker DJ. High-meat, low-carbohydrate diet in pregnancy: relation to adult blood pressure in the offspring. Hypertension 2001;38:1282-8.

37. Huh SY, Rifas-Shiman SL, Kleinman KP, Rich-Edwards JW, Lipshultz SE, Gillman MW. Maternal protein intake is not associated with infant blood pressure. Int J Epidemiol 2004; 34:378-84.

38. Belizan JM, Villar J, Bergel E, del Pino A, Di Fulvio S, Galliano SV, et al. Long-term effect of calcium supplementation during pregnancy on the blood pressure of offspring: follow up of a randomised controlled trial. BMJ 1997;315:281-5.

39. Hatton DC, Harrison-Hohner J, Coste S, Reller M, McCarron D. Gestational calcium supplementation and blood pressure in the offspring. Am J Hypertens 2003;16:801-5.

40. Gillman MW, Rifas-Shiman SL, Kleinman KP, Rich-Edwards JW, Lipshultz SE. Maternal calcium intake and offspring blood pressure. Circulation 2004;110:1990-5.

41. Morley R, Carlin JB, Dwyer T. Maternal calcium supplementation and cardiovascular risk factors in twin offspring. Int J Epidemiol 2004;33:1304-9.

42. Godfrey KM, Barker DJ. Fetal nutrition and adult disease. Am J Clin Nutr 2000;71:1344S-52S.

43. Smith GC, Pell JP, Walsh D. Pregnancy complications and maternal risk of ischaemic heart disease: a retrospective cohort study of 129,290 births. Lancet 2001;357:2002-6.

44. Mackenzie HS, Brenner BM. Fewer nephrons at birth: a missing link in the etiology of essential hypertension? Am J Kidney Dis 1995;26:91-8.

45. Martyn CN, Greenwald SE. Impaired synthesis of elastin in walls of aorta and large conduit arteries during early development as an initiating event in pathogenesis of systemic hypertension. Lancet 1997;350:953-5.

46. Hinchliffe SA, Lynch MR, Sargent PH, Howard CV, van Velzen D. The effect of intrauterine growth retardation on the development of renal nephrons. BJOG 1992;99:296-301.

47. Keller G, Zimmer G, Mall G, Ritz E, Amann K. Nephron number in patients with primary hypertension. N Engl J Med 2003;348:101-8.

48. Spencer J, Wang Z, Hoy W. Low birth weight and reduced renal volume in Aboriginal children. Am J Kidney Dis 2001;37:915-20.
49. Singh GR, Hoy WE. Kidney volume, blood pressure, and albuminuria: findings in an Australian aboriginal community. Am J Kidney Dis 2004;43:254-9.
50. Cheung YF, Wong KY, Lam BC, Tsoi NS. Relation of arterial stiffness with gestational age and birth weight. Arch Dis Child 2004;89:217-21.
51. Martyn CN, Barker DJ, Jespersen S, Greenwald S, Osmond C, Berry C. Growth in utero, adult blood pressure, and arterial compliance. Br Heart J 1995;73:116-21.
52. Seckl JR. Prenatal glucocorticoids and long-term programming. Eur J Endocrinol 2004;151: U49-62.
53. Schoof E, Girstl M, Frobenius W, Kirschbaum M, Dorr HG, Rascher W, et al. Decreased gene expression of 11-beta-hydroxysteroid dehydrogenase type 2 and 15-hydroxyprostaglandin dehydrogenase in human placenta of patients with preeclampsia. J Clin Endocrinol Metab 2001;86:1313-7.
54. Rogerson FM, Kayes KM, White PC. Variation in placental type 2 11beta-hydroxysteroid dehydroge-nase activity is not related to birth weight or placental weight. Mol Cell Endocrinol 1997;128:103-9.
55. Bocchi B, Kenouch S, Lamarre-Cliche M, Muffat-Joly M, Capron MH, Fiet J, et al. Impaired 11-beta hydroxysteroid dehydrogenase type 2 activity in sweat gland ducts in human essential hyperten-sion. Hypertension 2004;43:803-8.
56. Clark PM. Programming of the hypothalamo-pituitary-adrenal axis and the fetal origins of adult disease hypothesis. Eur J Pediatr 1998;157:S7-10.
57. Phillips DI, Walker BR, Reynolds RM, Flanagan DE, Wood PJ, Osmond C, et al. Low birth weight predicts elevated plasma cortisol concentrations in adults from 3 populations. Hypertension 2000;35:1301-6.
58. Ward AM, Syddall HE, Wood PJ, Chrousos GP, Phillips DI. Fetal programming of the hypothalamic-pituitary-adrenal (HPA) axis: low birth weight and central HPA regulation. J Clin Endocrinol Metab 2004;89:1227-33.
59. Pell JP, Smith GC, Walsh D. Pregnancy complications and subsequent maternal cerebrovascular events: a retrospective cohort study of 119,668 births. Am J Epidemiol 2004;159:336-42.
60. Lawlor DA, Davey Smith G, Ebrahim S. Birth weight of offspring and insulin resistance in late adult-hood: cross sectional survey. BMJ 2002;325:359-62.
61. Lindsay RS, Dabelea D, Roumain J, Hanson RL, Bennett PH, Knowler WC. Type 2 diabetes and low birth weight: the role of paternal inheritance in the association of low birth weight and diabetes. Diabetes 2000;49:445-9.
62. Yajnik CS, Coyaji KJ, Joglekar CV, Kellingray S, Fall C. Paternal insulin resistance and fetal growth: problem for the 'fetal insulin' and the 'fetal origins' hypotheses. Diabetologia 2001;44:1197-8.
63. Hypponen E, Smith GD, Power C. Parental diabetes and birth weight of offspring: intergenerational cohort study. BMJ 2003;326:19-20.
64. Yajnik CS, Joglekar CV, Pandit AN, Bavdekar AR, Bapat SA, Bhave SA, et al. Higher offspring birth weight predicts the metabolic syndrome in mothers but not fathers 8 years after delivery: the Pune Children's Study. Diabetes 2003;52:2090-6.
65. Wannamethee SG, Lawlor DA, Whincup PH, Walker M, Ebrahim S, Davey-Smith G. Birthweight of offspring and paternal insulin resistance and paternal diabetes in late adulthood: cross sectional survey. Diabetologia 2004;47:12-8.
66. Poulsen P, Vaag AA, Kyvik KO, Moller Jensen D, Beck-Nielsen H. Low birth weight is associated with NIDDM in discordant monozygotic and dizygotic twin pairs. Diabetologia 1997;40:439-46.
67. Poulter NR, Chang CL, MacGregor AJ, Snieder H, Spector TD. Association between birth weight and adult blood pressure in twins: historical cohort study. BMJ 1999;319:1330-3.
68. Dwyer T, Blizzard L, Morley R, Ponsonby AL. Within pair association between birth weight and blood pressure at age 8 in twins from a cohort study. BMJ 1999;319:1325-9.
69. Loos RJ, Fagard R, Beunen G, Derom C, Vlietinck R. Birth weight and blood pressure in young adults: a prospective twin study. Circulation 2001;104:1633-8.

70. Iliadou A, Cnattingius S, Lichtenstein P. Low birthweight and Type 2 diabetes: a study on 11,162 Swedish twins. Int J Epidemiol 2004;33:948-53.

71. RG IJ, Stehouwer CD, Boomsma DI. Evidence for genetic factors explaining the birth weight-blood pressure relation. Analysis in twins. Hypertension 2000;36:1008-12.

72. Christensen K, Stovring H, McGue M. Do genetic factors contribute to the association between birth weight and blood pressure? J Epidemiol Community Health 2001;55:583-7.

73. Johansson-Kark M, Rasmussen F, De Stavola B, Leon DA. Fetal growth and systolic blood pressure in young adulthood: the Swedish Young Male Twins Study. Paediatr Perinat Epidemiol 2002;16:200-9.

74. Hubinette A, Cnattingius S, Ekbom A, de Faire U, Kramer M, Lichtenstein P. Birthweight, early environment, and genetics: a study of twins discordant for acute myocardial infarction. Lancet 2001;357:1997-2001.

75. Vagero D, Leon D. Ischaemic heart disease and low birth weight: a test of the fetal-origins hypothesis from the Swedish Twin Registry. Lancet 1994;343:260-3.

76. Zhang J, Brenner RA, Klebanoff MA. Differences in birth weight and blood pressure at age 7 years among twins. Am J Epidemiol 2001;153:779-82.

77. Baird J, Osmond C, MacGregor A, Snieder H, Hales CN, Phillips DI. Testing the fetal origins hypothesis in twins: the Birmingham twin study. Diabetologia 2001;44:33-9.

78. Leon DA. The foetal origins of adult disease: interpreting the evidence from twin studies. Twin Res 2001;4:321-6.

79. Casteels K, Ong K, Phillips D, Bendall H, Pembrey M. Mitochondrial 16189 variant, thinness at birth, and type-2 diabetes. ALSPAC study team. Avon Longitudinal Study of Pregnancy and Childhood. Lancet 1999;353:1499-500.

80. Dunger DB, Ong KK, Huxtable SJ, Sherriff A, Woods KA, Ahmed ML, et al. Association of the INS VNTR with size at birth. ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. Nat Genet 1998;19:98-100.

81. Ong KK, Phillips DI, Fall C, Poulton J, Bennett ST, Golding J, et al. The insulin gene VNTR, type 2 diabetes and birth weight. Nat Genet 1999;21:262-3.

82. Bazaes RA, Petry CJ, Ong KK, Avila A, Dunger DB, Mericq MV. Insulin gene VNTR genotype is associated with insulin sensitivity and secretion in infancy. Clin Endocrinol 2003;59:599-603.

83. Ong KK, Petry CJ, Barratt BJ, Ring S, Cordell HJ, Wingate DL, et al. Maternal-fetal interactions and birth order influence insulin variable number of tandem repeats allele class associations with head size at birth and childhood weight gain. Diabetes 2004;53:1128-33.

84. Bennett AJ, Sovio U, Ruokonen A, Martikainen H, Pouta A, Taponen S, et al. Variation at the insulin gene VNTR (variable number tandem repeat) polymorphism and early growth: studies in a large Finnish birth cohort. Diabetes 2004;53:2126-31.

85. Mitchell SM, Hattersley AT, Knight B, Turner T, Metcalf BS, Voss LD, et al. Lack of support for a role of the insulin gene variable number of tandem repeats minisatellite (INS-VNTR) locus in fetal growth or type 2 diabetes-related intermediate traits in United Kingdom populations. J Clin Endocrinol Metab 2004;89:310-7.

86. Vaessen N, Heutink P, Janssen JA, Witteman JC, Testers L, Hofman A, et al. A polymorphism in the gene for IGF-I: functional properties and risk for type 2 diabetes and myocardial infarction. Diabetes 2001;50:637-42.

87. Vaessen N, Janssen JA, Heutink P, Hofman A, Lamberts SW, Oostra BA, et al. Association between genetic variation in the gene for insulin-like growth factor-I and low birthweight. Lancet 2002;359:1036-7.

88. Frayling TM, Hattersley AT, McCarthy A, Holly J, Mitchell SM, Gloyn AL, et al. A putative functional polymorphism in the IGF-I gene: association studies with type 2 diabetes, adult height, glucose tolerance, and fetal growth in U.K. populations. Diabetes 2002;51:2313-6.

89. Arends N, Johnston L, Hokken-Koelega A, van Duijn C, de Ridder M, Savage M, et al. Polymorphism in the IGF-I gene: clinical relevance for short children born small for gestational age. J Clin Endocrinol Metab 2002;87:2720-24.

90. Johnston LB, Dahlgren J, Leger J, Gelander L, Savage MO, Czernichow P, et al. Association between insulin-like growth factor I (IGF-I) polymorphisms, circulating IGF-I, and pre- and postnatal growth in two European small for gestational age populations. J Clin Endocrinol Metab 2003;88:4805-10.

91. Eriksson JG, Lindi V, Uusitupa M, Forsen TJ, Laakso M, Osmond C, et al. The effects of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-gamma2 gene on insulin sensitivity and insulin metabolism interact with size at birth. Diabetes 2002;51:2321-4.

92. Yliharsila H, Eriksson JG, Forsen T, Laakso M, Uusitupa M, Osmond C, et al. Interactions between peroxisome proliferator-activated receptor-gamma 2 gene polymorphisms and size at birth on blood pressure and the use of antihypertensive medication. J Hypertens 2004;22:1283-7.

93. Kennedy GC, German MS, Rutter WJ. The minisatellite in the diabetes susceptibility locus IDDM2 regulates insulin transcription. Nat Genet 1995;9:293-8.

94. Paquette J, Giannoukakis N, Polychronakos C, Vafiadis P, Deal C. The INS 5' variable number of tandem repeats is associated with IGF2 expression in humans. J Biol Chem 1998;273:14158-64.

95. Deeb SS, Fajas L, Nemoto M, Pihlajamaki J, Mykkanen L, Kuusisto J, et al. A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet 1998;20:284-7.

96. Frayling TM, Hattersley AT, Smith GD, Ben-Shlomo Y. Conflicting results on variation in the IGFI gene highlight methodological considerations in the design of genetic association studies. Diabetologia 2002;45:1605-6.

97. Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL. Children born small for gestational age: do they catch up? Pediatr Res 1995;38:267-71.

98. Forsen T, Eriksson J, Tuomilehto J, Reunanen A, Osmond C, Barker D. The fetal and childhood growth of persons who develop type 2 diabetes. Ann Intern Med 2000;133:176-82.

99. Eriksson JG, Forsen T, Tuomilehto J, Osmond C, Barker DJ. Early growth and coronary heart disease in later life: longitudinal study. BMJ 2001;322:949-53.

100. Bhargava SK, Sachdev HS, Fall CH, Osmond C, Lakshmy R, Barker DJ, et al. Relation of serial changes in childhood body-mass index to impaired glucose tolerance in young adulthood. N Engl J Med 2004;350:865-75.

101. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ 2000;320:967-71.

102. Stettler N, Zemel BS, Kumanyika S, Stallings VA. Infant weight gain and childhood overweight status in a multicenter, cohort study. Pediatrics 2002;109:194-9.

103. Singhal A, Cole TJ, Lucas A. Early nutrition in preterm infants and later blood pressure: two cohorts after randomised trials. Lancet 2001;357:413-9.

104. Singhal A, Fewtrell M, Cole TJ, Lucas A. Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. Lancet 2003;361:1089-97.

105. Hofman A, Jaddoe VW, Mackenbach JP, Moll HA, Snijders RF, Steegers EA, et al. Growth, development and health from early fetal life until young adulthood: the Generation R Study. Paediatr Perinat Epidemiol 2004;18:61-72.

106. Inskip HM, Godfrey KM, Robinson SM, Law CM, Barker DJ, Cooper C. Cohort profile: The Southampton Women's Survey. Int J Epidemiol 2006;35:42-8.

The Generation R Study

3

Rationale and design

3.1

Abstract

In this paper the Generation R Study is presented. This study examines growth, development and health in urban children from fetal life until young adulthood. With an integrated approach of epidemiological, clinical and basic research, this study focuses on four primary areas of research: 1) growth and physical development; 2) behavioural and cognitive development; 3) diseases in childhood; and 4) health and healthcare for pregnant women and children. The general aims of the study are:

1) To describe normal and abnormal growth, development and health from fetal life until young adulthood in a multi-ethnic population-based cohort;

2) To identify biological, social and environmental determinants of normal and abnormal growth, development and health from fetal life until adulthood;

3) To examine the effectiveness of current strategies for prevention and early identification of groups at risk.

Eventually, this study has to contribute to the development of strategies for optimizing health and healthcare for pregnant women and children. The Generation R Study is a population-based prospective cohort study in Rotterdam, the Netherlands. In this urban setting, 10,000 subjects are planned to be examined from early fetal life until young adulthood. Data are collected by physical examinations, questionnaires, interviews, ultrasound examinations and biological samples. The study entered its pilot phase in December 2001 with the inclusion of pregnant women. Full participant recruitment and complete data collection started in 2002.

General description

Introduction

Knowledge about growth, development and health in contemporary urban children is limited. Insights are primarily based on studies in diseased populations. However, small variations within the normal range of growth, development and health in fetal life and childhood may have life-long consequences for physical and mental health. These variations and their determinants can only be studied in large-scale population-based cohort studies. Current population-based cohort studies are not situated in large western cities and do not provide information on children from ethnic minorities [1].

Scope of research

The Generation R Study is designed to study growth, development and health in a contemporary population-based multi-ethnic cohort of urban children from fetal life until young adulthood. The study focuses on four primary areas of research: 1) growth and physical development; 2) behavioural and cognitive development; 3) diseases in childhood; and 4) health and healthcare for pregnant women and children. Special interest in these areas of research is on identification of early causal pathways leading to both normal and abnormal growth, development and health in childhood and adulthood. The general aims of the study are:

1) To describe normal and abnormal growth, development and health from fetal life until young adulthood in a multi-ethnic population-based cohort;
2) To identify biological, social and environmental determinants of normal and abnormal growth, development and health from fetal life until adulthood;
3) To examine the effectiveness of current strategies for prevention and early identification of groups at risk.

Eventually, this study has to contribute to the development of strategies for optimizing health and healthcare for pregnant women and children.

Rationale

The Generation R study is based on three main rationales. First, common physical and mental diseases in adult life seem to have at least part of their origin in early life. Risk factors for cardiovascular disease track from early childhood to adulthood [2, 3]. Social, emotional and physical deprivation in childhood are associated with psychopathology in adolescence and adult life [4, 5]. More recently, it has been suggested that in addition to the early postnatal phase, fetal life is also important for the development of diseases in later life [6, 7]. Causal pathways leading to common diseases in adult life may start in fetal life or infancy. Identification of the etiological determinants in fetal life and infancy

of common diseases in adult life is important for the development of future preventive strategies.

Second, the largest ethnic groups in Rotterdam are the Dutch, Moroccan, Surinamese and Turkish parents. Approximately 50% of the newborns in the large Dutch cities, including Rotterdam, have parents from ethnic minorities [8]. Knowledge about their growth, development and health is lacking. These children are not comparable with either their peers in their parents' countries because of the environmental effects or with their Dutch peers, because of the genetic differences. Evaluation of their growth, development and health will contribute to the development of specific strategies focused on the children of the largest ethnic groups.

Third, utilization of the preventive and curative health care system seems to differ between different social classes and ethnic groups [9]. Identifying determinants of health care utilization in pregnant women and children, especially in ethnic minorities, contributes to the development of new strategies and may lead to improved individual and community health.

Other population-based cohort studies like the Avon Longitudinal Study of Parents and Children (ALSPAC) have led to important contributions to knowledge about various health problems in pregnancy and childhood [1]. In addition to these studies, the Generation R Study will be focused in more detail on fetal growth and development and their determinants in the initial phase of the study, on ethnic specific health problems in childhood and current health care practices for pregnant women and children. As a consequence, not the size of the Generation R Study cohort but the ethnic variability will be larger and the measurements will be more detailed than in current studies.

Design

Overview

The Generation R Study is a population-based prospective cohort study on growth, development and health from early fetal life until young adulthood. Extensive assessments are carried out in pregnant women and their partners and children. Pregnant women are assessed at 12 (early pregnancy), 20 (mid-pregnancy) and 30 (late pregnancy) weeks of gestation to collect information about fetal growth and its main determinants. Their partners are assessed once (Tables 1-3). All measurements are performed in one of the two well-equipped research centers in Rotterdam. Postnatally, their children will participate in a prospective birth-cohort study until young adulthood. Data collection until the age of 4 years is carried out in the routine child health centers. Thereafter, the children will regularly be invited to one of the study centers.

Table 1. Planned assessments until the age of 4 years

Assessments in pregnant women
- Physical examinations: height, weight, blood pressure
- Questionnaires: socio-economic status, ethnicity, housing, living conditions, diet, medical history, family history, drug use, life style habits, use of medical services
- Interviews: expectations of future parents (only in focus cohort)
- Biological samples: blood samples, cord blood
- Ultrasound scans: gestational age, fetal growth and placental function and in the focus cohort fetal brain, heart, aorta and kidney development

Assessments in partners of pregnant women
- Physical examinations: height, weight, blood pressure
- Questionnaires: socio-economic status, ethnicity, housing, living conditions, medical history, family history, drug use, life style habits, use of medical services
- Interviews: expectations of future parents (only in focus cohort)
- Biological samples: blood samples

Assessments in children until the age of 4 years
- Physical examinations: length (height), weight, head circumference and in the focus cohort blood pressure, body composition and neurological development
- Questionnaires: diet, behaviour, living conditions, drug use, use of medical services
- Interviews: child-rearing practices (only in focus cohort)
- Ultrasounds: brain, heart, aorta and kidney development (only in focus cohort)
- Biological samples: blood samples, urine samples, saliva samples (only in focus cohort)

Table 2. Time scheme of assessments in mothers and their partners in pregnancy and their children at birth

	12 weeks	20 weeks	30 weeks	Birth
Mother				
Physical examination	+	+	+	
Questionnaire	+ +	+	+	
Interview			F	
Blood sample	+	+		
Ultrasound				
Gestational age	+			
Fetal growth		+	+	
Placenta function			F	
Fetal organ development			F	
Partner				
Physical examination	+			
Questionnaire		+		
Interview			F	
Blood sample	+			
Child				
Physical examination				+
Cord blood sample				+

(+ = assessment in whole cohort; F = assessment only in focus cohort)

Table 3. Time scheme assessments in children until the age of 48 months

	Age in months											
	1	2	3	4	6	9	11	14	18	24	36	48
Physical examination												
Anthropometrics	+	+	+	+	+	+	+	+	+	+	+	+
Body composition	F				F			F		F	F	F
Blood pressure	F				F					F		F
Motor development (Prechtl)	F		+		F			F				
Questionnaire		+			+			+		+	+	+
Interview					F							
Ultrasound												
Organ development	F				F					F		F
Biological samples												
Blood sample					F			F		F		F
Saliva sample					F			F		F	F	F

(+ = assessment in whole cohort; F = assessment only in focus cohort)

Focus Study

Additional detailed assessments of fetal and postnatal growth and development will be conducted in a subgroup of 1,000 children and their parents. This subgroup study is called the Generation R Focus Study. The aim of this study is to examine etiological associations with methods that cannot be used in the whole cohort. Research questions that will be addressed are related to early determinants of cardiovascular disease, diabetes and obesity, to determinants of infant maladjustment and to interactions between the immune system and infectious diseases. Measurements planned in the focus study are demonstrated in Tables 1-3.

Study cohort

The study cohort will consist of 10,000 children who meet the inclusion criteria (Table 4). To obtain prenatal information, all eligible pregnant women in Rotterdam and their partners are invited to participate. The number of newborns in Rotterdam is 7,000 per year. Based on a small pilot study, an overall response rate of 70% is considered as feasible. Assuming this response rate, two years are expected to be needed to form a cohort of 10,000 newborns. A vast part of this cohort will consist of ethnic minorities (Figure 1). Special efforts are undertaken to include the Dutch, other European, Moroccan, Surinamese and Turkish groups. Although the other ethnic groups are recruited as much as possible, the sample size and power of these ethnic groups are too small for all studies on ethnic differences.

Table 4. Eligibility criteria for pregnant women and inclusion criteria (at birth)

Eligibility criteria for pregnant women
Mother is expected to be resident in Rotterdam at date of delivery
Expected delivery date lies between June 2002 and July 2004
Legal resident of the Netherlands
Inclusion criteria at birth
Mother is resident in Rotterdam at date of delivery
Date of birth between June 2002 and July 2004
Informed consent by one of the parents
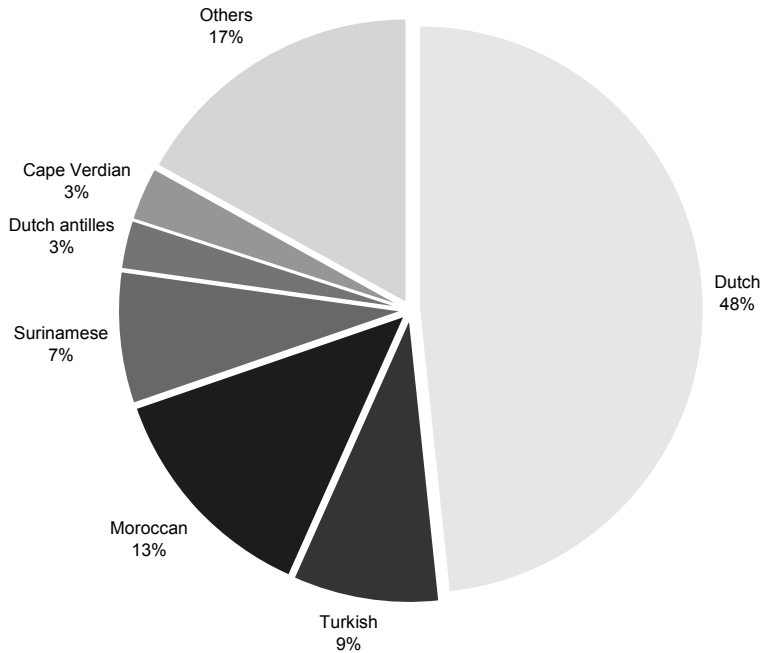
Figure 1. Ethnic distribution of newborns in Rotterdam
Based on children born in 2003 and 2004 (www.cos.rotterdam.nl)

Focus cohort

The focus cohort will consist of 1,000 Dutch children. Dutch is defined as two parents and four grandparents born in the Netherlands. An ethnic homogeneous group is chosen to exclude the confounding and effect modifying effect of ethnicity. As a consequence, research projects that study ethnicity as a determinant, outcome or effect modifier are not conducted within the focus cohort but in the whole Generation R cohort. All eligible parents whose children are expected to be born from April 2003 to April 2004 are invited to participate. Based on the pilot phase, a response rate of 80% in the focus cohort seems feasible.

Enrolment

Midwives and obstetricians give eligible pregnant women oral information about the study and hand out the information package. All eligible pregnant women who visit a midwife or obstetrician in Rotterdam are contacted by phone by the Generation R Study staff for additional information. For recruitment of pregnant women of ethnic minorities, translated information packages and questionnaires and study staff from these minority groups are available. The study staff is able to communicate with the pregnant women in Dutch, Arabic, English, French, Portuguese and Turkish. All pregnant women are asked to make an appointment for the first ultrasound. The study staff contacts these women again when they attend for their ultrasound for informed consent.

Informed consent

The study has been approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam. Pregnant women and their partners receive written and oral information about the study. Participants are asked for their written informed consent for the five consecutive phases of the study (prenatally, birth to 4 years, 4 to 12 years, 12 to 16 years, from 16 years onwards).

Main outcomes

All outcomes are related to one of the four research areas. These include: 1) growth and physical development; 2) behavioural and cognitive function; 3) diseases in childhood; and 4) health and healthcare utilization. These outcomes and research areas are described in more detail below.

Main determinants

Groups of determinants studied include:
–   Biological determinants: including parental anthropometrics, fetal and postnatal growth, endocrine and immunological factors and genetic variants related to growth.
–   Social determinants: including parental education, marital status, employment status, income level and ethnicity defined by country of birth of parents and grandparents.
–   Environmental determinants: including maternal and childhood diet, parental smoking, life style and housing.
These determinants are described in more detail below.

Assessments

A list of all assessments planned until the age of 4 years is shown in Tables 1-3. More detailed information presented in the Manual of Operations of the study is available upon request.

Logistics

The logistics of the study are carried out in close collaboration with the Stichting Trombosedienst Artsenlaboratorium Rijnmond, Rotterdam (STAR). The STAR collaborates in the recruitment of pregnant women and in all measurements in these women and their partners at the research centers. The logistics of the Generation R Study have been studied in the pilot phase and optimized thereafter.

Data quality, control and management

The Generation R Study staff and the STAR staff are regularly centrally trained and are required to demonstrate competency in relevant procedures before doing procedures for the Generation R Study. These procedures include anthropometric and blood pressure measurements, venous punctures, specimen processing, ultrasound examinations and data entry. All measurements are checked by examination of the data including the means, standard deviations and data outliers and their differences between staff members. Data on intra- and inter-observer reproducibility of the ultrasound scans are obtained by studies in small subgroups. Data collected at the research centers are entered onto forms and into the electronic database. All principal investigators have access to their own data for read-only purposes. After data collection is complete, the data are cleaned and distributed to the principal investigators for analyses.

Biological samples

All biological samples collected in the Generation R Study are stored at the STAR. The amount of venous blood taken is 35 and 20 ml at 12 and 20 weeks, respectively, from the mother, 10 ml from her partner and 20 ml from the newborn (cord blood). These plasma and serum samples are distributed in small aliquots (each 250 μl), and stored at –80°C. DNA is extracted from whole blood samples. A urine sample is collected and stored for future measurements.

Time schedule

The Generation R Study entered its pilot phase in December 2001. Full participant recruitment and complete data collection started in 2002. A cohort of 10,000 newborns is expected to be completed in 2004. The whole study is expected to run until all children have reached the age of 20 years.

Research projects

According to the main outcome measures, all research projects in the Generation R Study are embedded in one of the four research areas: 1) growth and physical development; 2) behavioural and cognitive function; 3) diseases in childhood; and 4) health and healthcare utilization. A description of the specific aims, measurements and projects in these research areas is given here in detail.

Growth and physical development

Main research aims
1) To identify biological, social and environmental determinants of normal and abnormal growth and physical development patterns;
2) To identify fetal and infant determinants of risk factors for cardiovascular disease, diabetes and obesity.

Specific measurements planned until the age of 4 years
– Parental physical examination (whole cohort): height, weight, blood pressure;
– Fetal growth (whole cohort): gestational age (crown-rump length, biparietal diameter at 10-12 weeks of gestation), fetal growth (biparietal diameter, head circumference, abdominal circumference, femur length at 20 and 30 weeks of gestation);
– Fetal blood flow distribution and haemodynamic placenta function (focus cohort): pulsatility index in the abdominal aorta, medial cerebral artery, uterine artery and umbilical artery (30 weeks of gestation);
– Postnatal growth (whole cohort): length (height), weight, head circumference (at 1, 2, 3, 4, 6, 9, 11, 14, 18, 24, 36 and 48 months);
– Motor development: Prechtl scales (whole cohort at 3 months, focus cohort at 1, 6, 14 months);
– Body composition (focus cohort): Skinfold thickness (at 1, 6, 14, 24, 36 and 48 months), dual energy X absorptiometry-scan (at 6, 24 and 48 months);
– Nutrition (whole cohort): Maternal diet (at 12 weeks of gestation) and childhood diet (2, 6, 14 and 24 months and yearly thereafter) by food frequency questionnaires;
– Risk factors for cardiovascular disease (focus cohort): Blood pressure (at 1, 6, 24, and 48 months), blood lipids (at birth, 6, 24 and 48 months), left ventricular function and renal size (at 30 weeks prenatally and 1, 6, 24 and 48 months postnatally), left ventricular mass (at 1, 6, 24 and 48 months);
– Risk factors for type 2 diabetes (focus cohort): insulin-glucose ratios (at 6 and 24 months), oral glucose tolerance tests (at 48 months);

– Genetic variations (focus cohort): polymorphisms related to insulin, insulin-like growth factors, growth hormone and cortisol activity.

Specific projects

Fetal and postnatal growth patterns
There is incomplete knowledge about determinants of fetal and postnatal growth. As small variations in fetal and early postnatal growth may have life long consequences, it is important to obtain insight in the determinants of normal and abnormal fetal and postnatal growth patterns [6]. The following research questions will be addressed: 1) What are normal and abnormal fetal and postnatal growth patterns in different ethnicities? 2) What are the biological, social and environmental determinants of these growth patterns?

Ethnic differences in birth outcomes
Perinatal death, low birth weight and preterm birth seem to be more prevalent in lower socio-economic status and ethnic minority groups [10, 11]. Insight in the underlying factors that lead to adverse birth outcomes and pregnancy complications like pre-eclampsia in these groups is important for the development of group specific preventive strategies. Research questions that will be addressed include: 1) What is the prevalence of low birth weight and preterm birth in different ethnic and socio-economic groups? 2) What is the prevalence of specific determinants of these adverse birth outcomes in these different groups? 3) Do these specific determinants underlie the differences in birth outcomes?

Early determinants of obesity
Childhood obesity is a major health problem and seems to be predictive for obesity, cardiovascular disease and diabetes in adulthood [12]. This research project aims at getting insight in the prevalence, determinants and consequences of childhood obesity. The following research questions will be addressed: 1) What is the incidence of obesity in childhood? 2) What are the biological, social and behavioural determinants and consequences of childhood obesity? Biological determinants of special interest will be on endocrine factors and genetic variants related to insulin, insulin-like growth factors, leptin and cortisol activity.

Fetal and infant origins of cardiovascular disease and type 2 diabetes
Small size at birth is associated with cardiovascular disease and type 2 diabetes in adult life [13, 14]. Fetal life and infancy may be critical periods for development of these diseases. This research project aims at studying these associations and the underlying

mechanisms. The following research questions will be addressed: 1) Which fetal and infant growth patterns are associated with the development of risk factors for cardiovascular disease and type 2 diabetes? 2) Which environmental factors and genetic variants underlie these associations? This project will also study the development of risk factors for cardiovascular disease and type 2 diabetes from birth until young adulthood.

Behavioural and cognitive development

Main research aims

1) To identify biological, social and environmental determinants of normal and abnormal behavioural and cognitive development;
2) To study the long-term consequences of abnormal behavioural and cognitive development in infancy and early childhood.

Specific measurements planned until the age of 4 years

– Infant behaviour (whole cohort): mother and baby scales, feeding, crying and sleeping by questionnaires (Infant Behavior Questionnaire); problem behaviour by the Child Behavior Checklist (at 2, 6, 14, 24, 36 and 48 months);
– Child cognitive function: questionnaires (whole cohort, at 6, 14, 24, 36 and 48 months) and cognitive function tests (focus cohort, at 24 and 48 months);
– Parental psychopathology: Brief Symptom Inventory (whole cohort, at 20 weeks of gestation and 2 months), Edinburgh Postnatal Depression Scale (whole cohort, 2 months), Composite International Diagnostic Interview, Family Informant Schedule Criteria and 3 Minutes Speech Sample (focus cohort, at 30 weeks of gestation and 6 months);
– Child rearing: questionnaires about grand-parental rearing practices, parental childhood experiences, parental attitudes regarding child rearing practices (whole cohort, at 20 weeks of gestation) and about child rearing (whole cohort, at 2, 6, 14, 24, 36 and 48 months). Mother-child observation instruments (focus cohort, at 6 and 24 months);
– Child stress reactivity (focus cohort): saliva cortisol (at 6, 14, 24, 36 and 48 months);
– Maternal thyroid function (focus cohort): total thyroxine, free thyroxine, thyroid stimulating hormone (12 and 20 weeks of gestation). Total iodine in urine samples collected in early pregnancy;
– Fetal and infant brain development (focus cohort): ultrasound measured ventricular diameter, transcerebellar diameter (at 30 weeks of gestation), corpus callosum, ventricular diameter and brain volume (at 1 month).

Specific projects

Infant maladjustment and problem behaviour
Knowledge about the determinants and consequences of abnormal social and emotional behavioural functioning in infancy and childhood is notably lacking. Parental psychopathology may lead to problem behaviour in the offspring [15]. However, it is not known how this is related to parent-child interaction, adverse events and biological factors that influence infant and child behaviour. Research questions that will be addressed include: 1) What are the prenatal and early postnatal determinants of infant maladjustment and childhood problem-behaviour? 2) What are the long-term consequences of problem behaviour in infancy and childhood on social, emotional and intellectual development? Determinants that will be studied include parental psychopathology, child-rearing practices and the child's stress-reactivity, temperament and brain development.

Cognitive functioning
Acquisition of knowledge and skills is essential for the growing and developing child. Early factors seem to be important determinants of cognitive functioning [7]. Impaired cognitive function in childhood may have life long consequences. The following research questions will be addressed: 1) What are the early determinants of impaired cognitive function in childhood? 2) What are the long-term consequences of impaired cognitive function in childhood? Early determinants that will be studied include socio-economic status, ethnicity, child-rearing practices, brain development, cortisol levels and maternal thyroid function.

Diseases in childhood

Main research aims

1) To study the incidence rates of common diseases and accidents in childhood;
2) To identify the biological, social and environmental determinants and long-term consequences of these diseases and accidents.

Common diseases in childhood of interest include infectious diseases, asthma and neurological paroxysmal events.

Specific measurements planned until the age of 4 years

– Diseases (whole cohort): questionnaires and the general practitioners register for information on infectious diseases, asthma, paroxysmal events and other diseases (at 2, 6, 14, 24, 36 and 48 months);
– Infectious serology (focus cohort): serology of several respiratory infectious diseases determined (at 6, 14, 24 and 48 months);

- Vaccination status (focus cohort): responses to Haemophilus influenzae B and meningococcal C vaccination (at 6 and 14 months) and measles vaccination (at 14 months);
- Immune system (focus cohort): immunophenotyping of T and B lymphocytes, NK cells and their subpopulations, maturation and activation of lymphocyte sub-populations, immunoglobuline G and subclasses, A, M and E (at birth, 6, 14 and 24 months);
- Lung function (focus cohort): static and dynamic lung function (at 48 months).

Specific projects

Infectious diseases and immunology
Infectious diseases are the most frequent acute diseases in childhood. The clinical response on infectious diseases varies between subjects. Genetic and environmental variations seem to be important determinants of clinical responses on infectious diseases [16, 17]. This project will address the following research questions: 1) What is the incidence of clinical and sub-clinical infectious diseases in childhood? 2) What are the genetic and environmental determinants of differences in clinical responses on infectious diseases. Special focus in this study is on the interactions between components of the immune system and respiratory infectious diseases.

Asthma
Asthma or asthma related symptoms are frequently seen health problems in infancy and childhood with consequences for later life [18]. This research project is designed to study the frequency, determinants and consequences of childhood asthma or asthma related symptoms. Special interest is on differences between ethnic groups. Research questions include: 1) What is the incidence of asthma and asthma related symptoms from birth until young adulthood? 2) What are the environmental and genetic determinants of these diseases? Development of the immune system, studied in the research project on infectious diseases and immunology, is also being studied in relation to asthma and asthma-related symptoms.

Paroxysmal neurological disorders
The incidence of convulsive or paroxysmal disorders in Dutch children is unknown. Determinants and consequences of paroxysmal disorders and febrile seizures in the Dutch population have not been studied in a population-based study. Research questions in this project include: 1) What is the incidence of seizures, febrile convulsions and other paroxysmal events? 2) What are the environmental and genetic determinants and long-term consequences of these events? More detailed information is collected in the

affected children by questionnaires and by electro-encephalograms. The affected children, their siblings and parents will participate in a study to examine genetic determinants of paroxysmal disorders.

Health and healthcare

Main research aims

1) To identify determinants of quality of life from childhood until young adulthood;
2) To identify determinants of health care utilization in pregnancy, infancy and childhood;
3) To examine the effectiveness of current and recently developed screening programs.

Specific measurements planned until the age of 4 years

– Quality of life (whole cohort): questionnaires in childhood;
– Health care utilization (whole cohort): registries of midwifery practices, obstetric departments in hospitals, child health centers and paediatric departments;
– Effectiveness of screening programmes (whole cohort): congenital malformations, development, congenital heart disease, cryptorchidism and development of speech.

Specific projects

Quality of life
Among adults, health-related quality of life is seen as an important outcome measure for health care interventions. Also, health status measures are important determinants of health care utilization. Much less is known about health-related quality of life in children. This project will address the following research questions: 1) What are the biological, social and psychological determinants of health-related quality of life in children? 2) What are the consequences of impaired health-related quality of life among children in terms of health care utilization and social participation?

Health care utilization
Utilization of the health care system differs between different ethnic and socio-economic status groups [19]. Research questions in this project include: 1) What is the health care utilization in pregnancy and in childhood? 2) What are the social, cultural and emotional determinants of the differences in healthcare utilization?

Effectiveness of current and new screening programs

Screening and vaccination programmes are performed at the child health centers, which are part of the preventive youth health care system [11]. This project addresses the following research question: What is the cost-effectiveness of current and recently developed screening programmes? These programmes are focused on neonatal hearing, asthma-related symptoms and development of speech in ethnic minorities. The Generation R Study is an observational and not an experimental study. Therefore, this sub-study will be observational, resulting in comparisons with an earlier cohort (before the start of the Generation R Study) and in descriptives about utilization differences between ethnic or socio-economic groups.

References

1. Golding J, Pembrey M, Jones R. ALSPAC: the Avon Longitudinal Study of Parents and Children. I. Study methodology. Paediatr Perinat Epidemiol 2001;15:74-87.
2. Bao W, Threefoot SA, Srinivasan SR, Berenson GS. Essential hypertension predicted by tracking of elevated blood pressure from childhood to adulthood: the Bogalusa Heart Study. Am J Hypertens 1995;8:657-65.
3. Webber LS, Srinivasan SR, Wattigney WA, Berenson GS. Tracking of serum lipids and lipoproteins from childhood to adulthood. The Bogalusa Heart Study. Am J Epidemiol 1991;133:884-99.
4. Hofstra MB, van der Ende J, Verhulst FC. Child and adolescent problems predict DSM-IV disorders in adulthood: a 14-year follow-up of a Dutch epidemiological sample. J Am Acad Child Adolesc Psychiatry 2002;41:182-9.
5. Ferdinand RF, Verhulst FC. Psychopathology from adolescence into young adulthood: an 8-year follow-up study. Am J Psychiatry 1995;152:1586-94.
6. Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet 1986;1:1077-81.
7. Richards M, Hardy R, Kuh D, Wadsworth ME. Birthweight, postnatal growth and cognitive function in a national UK birth cohort. Int J Epidemiol 2002;31:342-8.
8. Statistics Netherlands. Allochtonen in Nederland 2001.
9. Burgmeijer RJF, Merkx JAM. (ed.) Pakket ... en hoe pakt het uit? Ouder- en kindzorg tussen wetenschap en praktijk. Nederlands Congres Ouder- en Kindzorg. Assen: van Gorcum1999.
10. Shiono PH, Klebanoff MA, Graubard BI, Berendes HW, Rhoads GG. Birth weight among women of different ethnic groups. JAMA 1986;255:48-52.
11. Demissie K, Rhoads GG, Ananth CV, Alexander GR, Kramer MS, Kogan MD, Joseph KS. Trends in preterm birth and neonatal mortality among blacks and whites in the United States from 1989 to 1997. Am J Epidemiol 2001;154:307-15.
12. Whitaker RC, Wright JA, Pepe MS, Seidel KD, Dietz WH. Predicting obesity in young adulthood from childhood and parental obesity. N Engl J Med 1997;337:869-73
13. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. Lancet 1989;2:577-80.
14. Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C, Winter PD. Fetal and infant growth and impaired glucose tolerance at age 64. BMJ 1991;303:1019-22.

15. Lieb R, Isensee B, Hofler M, Pfister H, Wittchen HU. Parental major depression and the risk of depression and other mental disorders in offspring: a prospective-longitudinal community study. Arch Gen Psychiatry 2002;59:365-74.

16. Oppenheimer SJ. Iron and its relation to immunity and infectious disease. J Nutr 2001;131:616S-633S

17. van der Pol W, van de Winkel JG. IgG receptor polymorphisms: risk factors for disease. Immunogenetics 1998;48:222-32.

18. Stein RT, Sherrill D, Morgan WJ, Holberg CJ, Halonen M, Taussig LM, Wright AL, Martinez FD. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. Lancet 1999;354:541-5.

19. Stronks K, Ravelli AC, Reijneveld SA. Immigrants in the Netherlands: equal access for equal needs? J Epidemiol Community Health 2001;55:701-7

Cohort profile

3.2

Abstract

The Generation R Study is a population-based prospective cohort study from fetal life until young adulthood. The study is designed to identify early environmental and genetic causes of normal and abnormal growth, development and health from fetal life until young adulthood. The study focuses on four primary areas of research: 1) growth and physical development; 2) behavioural and cognitive development; 3) diseases in childhood; and 4) health and healthcare for pregnant women and children. In total, 9,778 mothers with a delivery date from April 2002 until January 2006 were enrolled in the study. Of all eligible children at birth, 61% participate in the study. Data collection in the prenatal phase included physical examinations, questionnaires, fetal ultrasound examinations and biological samples. In addition, more detailed assessments are conducted in a subgroup of 1,232 pregnant women and their children. The children form a prenatally recruited birth-cohort that will be followed until young adulthood. Eventually, results forthcoming from the Generation R Study have to contribute to the development of strategies for optimizing health and healthcare for pregnant women and children.

Introduction

The Generation R Study is a population-based prospective cohort study from fetal life until young adulthood. The background and specific research projects of the study have been described in detail previously [1]. This paper focuses on the study design and cohort profile in the first phase of the study.

Scope of research

The Generation R Study is designed to identify early environmental and genetic causes of normal and abnormal growth, development and health from fetal life until young adulthood. The study focuses on four primary areas of research: 1) growth and physical development; 2) behavioural and cognitive development; 3) diseases in childhood; and 4) health and healthcare for pregnant women and children. The main outcomes and determinants are presented in Tables 1 and 2. The general aims of the study are:
1) To describe normal and abnormal growth, development and health from fetal life until young adulthood;
2) To identify biological, environmental and social determinants of normal and abnormal growth, development and health from fetal life until young adulthood;
3) To examine the effectiveness of current strategies for prevention and early identification of groups at risk.

Eventually, results from the Generation R Study will contribute to the development of strategies for optimizing health and healthcare for pregnant women and children.

Study area

The Generation R Study is conducted in Rotterdam, the second largest city in the Netherlands. Rotterdam is situated in the Western part of the Netherlands on almost 80 kilometers south from Amsterdam, the capital of the Netherlands. The total population consists of about 600,000 inhabitants of almost 150 different ethnicities. The study area is well defined by postal codes and covers more than half of the cities inhabitants (almost 350,000 inhabitants). The largest ethnic groups in this population are the Dutch (56%), Surinamese (9%), Turkish (7%), Moroccan (6%), Dutch Antillean (3%) and Cape Verdian (3%) groups [2]. The percentages of the non-Dutch groups are higher in younger age groups [2]. The number of children born in this study area is about 4,300 per year [2]. The ethnic distribution of the newborns is shown in Figure 1. Measurements are conducted in two well-equipped research centers in the study area. Intensive collaboration was

established with all eight midwifery practices, three hospitals and sixteen child health centers located in this area.

Table 1. Main outcomes per research area

Growth and physical development
– Fetal growth patterns and organ development
– Pregnancy complications
– Postnatal growth patterns
– Obesity
– Risk factors for development of cardiovascular disease
– Risk factors for type 2 diabetes

Behaviour and cognitive development
– Maternal and paternal psychopathology
– Fetal and postnatal brain development
– Behaviour, psychopathology and cognition
– Neuromotor development
– Chronic pain
– Attachment
– Stress reactivity

Diseases in childhood
– Infectious diseases in childhood
– Development of the immune system
– Asthma and asthma related symptoms
– Paroxysmal neurological disorders

Health and healthcare
– Quality of life
– Health care utilization
– Effectiveness of screening programmes

Table 2. Main determinants

Biological determinants
– Parental anthropometrics and blood pressure
– Fetal and postnatal growth characteristics
– Endocrine and immunological factors
– Genetic variants

Environmental determinants
– Maternal and childhood diet
– Parental life style habits (including smoking, alcohol consumption)
– Housing conditions

Social determinants
– Parental education, employment status and household income
– Parental marital status
– Ethnicity
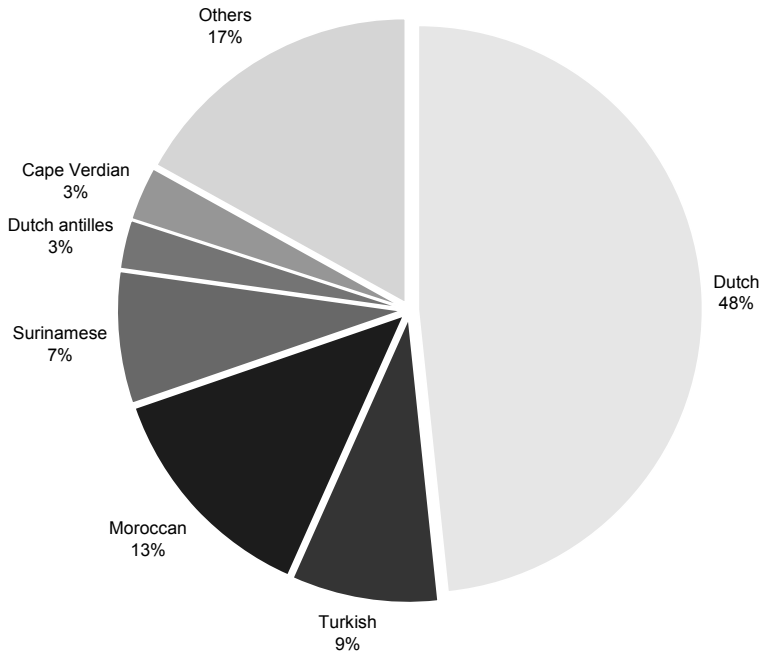
Figure 1. Ethnic distribution of newborns in Rotterdam
Based on children born in 2003 and 2004 (www.cos.rotterdam.nl)

Study design

Overview

The Generation R Study is a population-based prospective cohort study from fetal life until young adulthood. Mothers with a delivery date between April 2002 and January 2006 were eligible. Extensive assessments were carried out in mothers and their partners in pregnancy and are currently performed in their children (Table 3). Assessments in pregnancy were planned in early pregnancy (gestational age < 18 weeks), mid-pregnancy (gestational age 18 - 25 weeks) and late pregnancy (gestational age ≥ 25 weeks). The individual time scheme of these assessments depended on the specific gestational age at enrolment (Tables 4 and 5). The partners were assessed once in pregnancy. The children form a prenatally recruited birth-cohort that will be followed until young adulthood. Additionally, more detailed assessments of fetal and postnatal growth and development are conducted in a randomly selected subgroup of Dutch children and their parents, referred to as the Generation R Focus Cohort. This subgroup is ethnic homogeneous to exclude possible confounding or effect modification by ethnicity. Studies conducted in this subgroup examine etiological associations with more in-depth

Table 3. Assessments in the prenatal phase

Assessments in mothers
- Physical examinations: height, weight, blood pressure
- Questionnaires: socio-economic status, ethnicity, housing, living conditions, diet, medical history, family history, drug use, life style habits, use of medical services
- Interviews: expectations of parents to be (only in focus cohort)
- Biological samples: blood and urine samples (storage, DNA)
- Fetal ultrasounds: gestational age, fetal growth and in the focus cohort fetal brain, heart and kidney development, fetal blood flow distribution and placental function

Assessments in partners
- Physical examinations: height, weight, blood pressure
- Questionnaires: socio-economic status, ethnicity, housing, living conditions, medical history, family history, drug use, life style habits, use of medical services
- Interviews: expectations of parents to be (only in focus cohort)
- Biological samples: blood samples (storage, DNA)

Assessments in newborns at birth
- Physical examinations: weight
- Cord blood sample (storage, DNA)

Table 4. Assessments in mothers, their partners and their children in the prenatal phase

	Early pregnancy	Mid-pregnancy	Late pregnancy	Birth
Mother				
Physical examination	+	+	+	
Questionnaire	++	+	+	
Interview			F	
Fetal ultrasound examination	+	+	+	
Additional detailed fetal ultrasound			F	
Blood sample	+	+		
Urine sample	+	+	+	
Partner				
Physical examination	+			
Questionnaire		+		
Interview			F	
Blood sample	+			
Child				
Physical examination				+
Cord blood sample				+

(+ = assessment in whole cohort; F = assessment only in focus cohort)

Early pregnancy: gestational age < 18 weeks; mid-pregnancy: gestational age 18 - 25 weeks; late pregnancy: gestational age ≥ 25 weeks.

Table 5. Planned time scheme of prenatal measurements in mothers (weeks)

Gestational age at enrolment	Postal questionnaires (see text)				Visits for measurements		
	Mother 1	Mother 2	Mother 3	Mother 4	Early pregnancy	Mid-pregnancy	Late pregnancy
<12 weeks	12	15	20	30	12	20	30
13 weeks	13	15	20	30	13	20	30
14 weeks	14	16	20	30	14	20	30
15 weeks	15	17	20	30	15	20	30
16 weeks	16	18	22	30	16	22	30
17 weeks	17	19	22	30	17	22	30
18 weeks	18	20	24	30	-	18	30
19 weeks	19	21	24	30	-	19	30
20 weeks	20	22	24	30	-	20	30
21 weeks	21	23	25	30	-	21	30
22 weeks	22	24	26	30	-	22	30
23 weeks	23	25	27	30	-	23	30
24 weeks	24	-	-	30	-	24	30
≥ 25 weeks	≥ 25	-	-	≥ 30	-	-	≥ 25
Postnatal	Postnatal	-	-	-	-	-	-

Early pregnancy: gestational age < 18 weeks; mid-pregnancy: gestational age 18 - 25 weeks; late pregnancy: gestational age ≥ 25 weeks.

methods that cannot be applied in the whole cohort due to time, financial or logistical constraints. The Generation R Study is approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam. Mothers and their partners received written and oral information about the study. Participants are asked for their written informed consent for the five consecutive phases of the study (prenatally, birth to 4 years, 4 to 12 years, 12 to 16 years and from 16 years onwards).

Eligibility and enrolment

Eligible mothers were those who were resident in the study area at their delivery date and had a delivery date from April 2002 until January 2006. We aimed to enroll mothers in early pregnancy (gestational age < 18 weeks) but enrolment was possible until birth of their child. Midwives and obstetricians informed eligible mothers about the study at their first prenatal visit in routine care, handed out the information package and asked these mothers to make an appointment for the first ultrasound examination. The study staff contacted these mothers by phone for additional information about the study and in person at the ultrasound examination to obtain informed consent. Based on the pilot phase, it was estimated that it was possible to contact about 80% of all eligible mothers in pregnancy and 70% of these mothers would be willing to participate in the study. Mothers who were not approached in pregnancy, were approached and enrolled in the first months after birth of their child when newborns visit the routine child health

centers [3]. The partners were not approached directly by the study staff but the mothers were informed about the importance of involvement of their partners in the study. There was no specific definition of being a partner but information was obtained about the biological relation of the partner with the (unborn) child.

Eligibility criteria for enrolment in the Generation R Focus Study were enrolment before a gestational age of 25 weeks in the Generation R Study, Dutch ethnicity, defined as two parents and four grandparents born in the Netherlands and a delivery date between February 2003 and August 2005. The study staff contacted these eligible mothers in pregnancy by phone to inform them about this sub-study and to invite them for the first additional measurements at a gestational age of 30 weeks.

Pilot phase

The Generation R Study entered its pilot phase in December 2001 with the recruitment of pregnant women from the whole city of Rotterdam. The main aim of this pilot phase was to test the logistics of the enrolment process. Based on the logistic results from this pilot phase, the definite study area was limited to postal codes covering the largest part but not the whole city.

In the pilot phase, enrolment of mothers was restricted to a maximal gestational age of 24 weeks at enrolment. Since it turned out to be not feasible to approach all mothers in early or mid-pregnancy, the enrolment period for mothers was subsequently extended to the whole pregnancy until birth of their child. Full participant recruitment in the definite study area was established for mothers with a delivery date from January 2003. Mothers enrolled in the pilot phase and living outside the definite study area at their delivery date were completely followed until birth of their child. The children of these mothers do not participate in postnatal follow-up studies. Until the end of pregnancy, data collection and quality in these mothers are similar to data collected in the other participants. Therefore, these mothers are part of the total cohort for research projects studying outcomes in pregnancy. All enrolled mothers living in the definite study area at delivery with a delivery date from April 2002 and January 2006 are included as participants in the cohort for both prenatal and postnatal follow-up studies. Separate pilot studies to assess the intra- and inter-observer reproducibility were conducted in subjects outside the cohort and were, if necessary, repeated in study participants.

Study cohort

Pregnant women and their partners

In total, 9,778 mothers were enrolled in the study (Figure 2). Of these mothers, 91% (n = 8,880) was enrolled in pregnancy. Only partners from mothers enrolled in preg-

nancy were invited to participate. In total, 71% (n = 6,347) of all partners were enrolled. The general characteristics of the mothers and their partners are presented in Table 6. Of all participating mothers, enrolment was in early pregnancy in 69% (n = 6,748), in mid-pregnancy in 19% (n = 1,857), in late pregnancy in 3% (n = 275) and at birth of their child in 9% (n = 898). Of all mothers enrolled in pregnancy, 94% (n = 8,356), 6% (n = 516) and

Enrolment

Mothers: 9,778

(8,880 in pregnancy, 898 at birth of their child)

Partners: 6,347

Data collection in mothers (n = 8,880)

Visits
- Early pregnancy: 76% (n = 6,748)
- Mid-pregnancy: 93% (n = 8,241)
- Late pregnancy: 93% (n = 8,270)

Blood samples
- Early pregnancy: 72% (n = 6,398)
- Mid-pregnancy: 86% (n = 7,616)

Questionnaires
- Mother 1:   91% (n = 8,116)
- Mother 2:   81% (n = 7,229)
- Mother 3:   80% (n = 7,145)
- Mother 4:   77% (n = 6,830)

Data collection in partners (n = 6,347)

Prenatal visits: 100% (n = 6,347)
Blood samples: 84% (n = 5,326)
Questionnaires: 82% (n = 5,177)

1,195 mothers living outside definite study area and excluded from postnatal follow-up

Live births   98%
Induced abortion   0.3%
Fetal, perinatal losses   1.1%
Loss to follow-up (prenatally)   0.7%

Expected number of mothers and children eligible

for postnatal follow-up studies
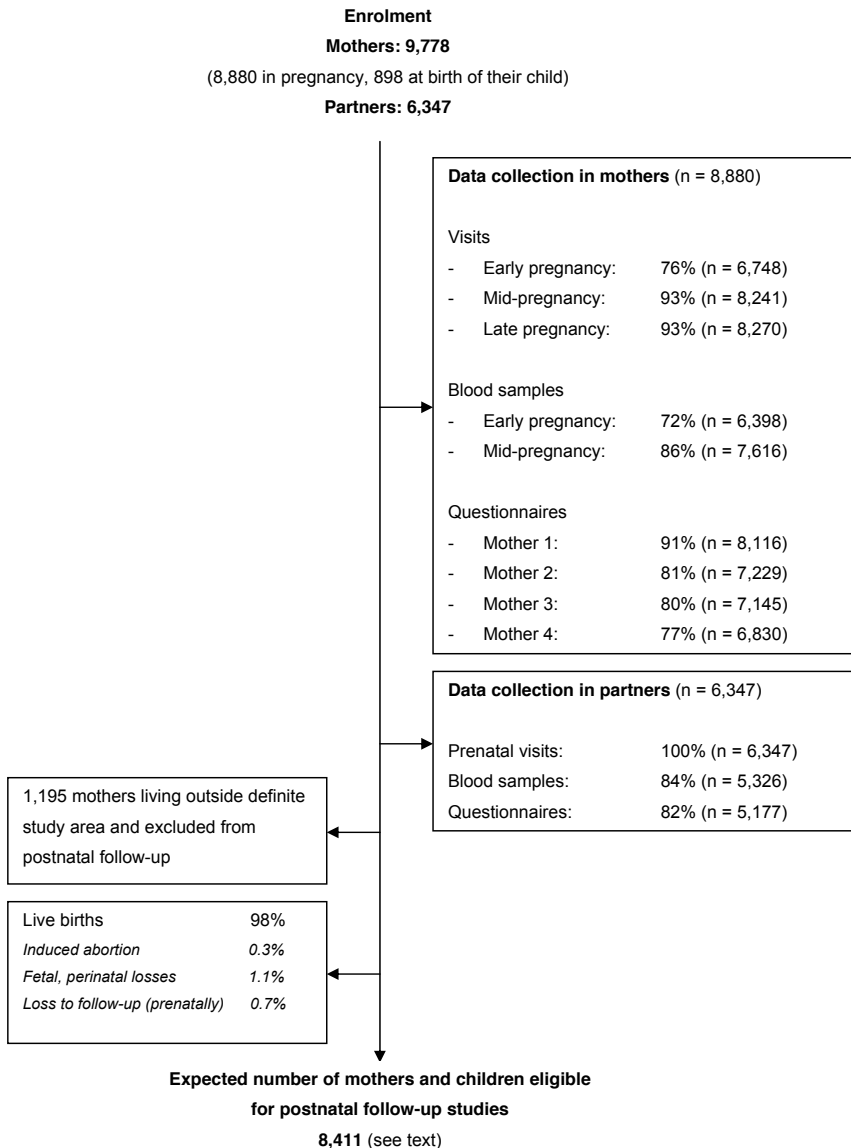
8,411 (see text)

Figure 2. Enrolment and measurements in the first phase

Table 6. General characteristics of the mothers and their partners

	Mothers	Partners
	(n = 9,778)	(n = 6,347)
Gestational age at enrolment (%)		
Early pregnancy	69	-
Mid-pregnancy	19	-
Late pregnancy	3	-
Birth	9	-
Pregnancy number in study (%)		
1st pregnancy	94	-
2nd pregnancy	6	-
3rd pregnancy	0.1	-
Age (years)	29.7 (5.3)	32.7 (5.8)
Parity (%)		
0	55	-
1	31	-
≥ 2	14	-
Ethnicity (%)		
Dutch, other-European	58	70
Surinamese	9	6
Moroccan	7	4
Turkish	9	6
Dutch Antilles	4	3
Cape Verdian	4	2
Others	9	9
Educational level (%)		
Primary school	13	8
Secondary school	45	41
Higher education	42	51
Household income per month (%)		
<€ 800	9	-
€ 800-2200	36	-
>€ 2200	55	-

Values are percentages. *Mean (standard deviation)
All subject characteristics, except gestational age at enrolment, are based on valid data in mothers who were enrolled in pregnancy.

0.1% (n = 8) were first, second and third pregnancies in the study, respectively. A total of 1,232 mothers were enrolled in the Generation R Focus Study. The mean age of mothers in our study at enrolment was similar to the age of all pregnant women in the study area (29.6 years in 2003) [2]. Ethnicity of participating mothers and partners was defined according the classification of Statistics Netherlands [4]. This means for one specific person that: 1) if both parents are born in the Netherlands, the ethnicity is Dutch; 2) if one of the parents is born in another country than the Netherlands, that country counts; 3)

if both parents are born in the same country other than the Netherlands, that country counts; 4) if the parents are born in the different countries other than the Netherlands, the country of mothers counts; and 5) if that person and both parents are born in different countries other than the Netherlands, the country of birth of that specific person counts. As expected, the largest ethnic groups were the Dutch, Surinamese, Turkish and Moroccan groups. The ethnic distribution differed only moderately from that of the population in the study area [2]. Mean household income in Rotterdam is about € 1,600 per month and the percentage subjects with a secondary or higher education level in Rotterdam is 56% [2]. The educational level of participating mothers and their partners was classified in groups according to the classification of Statistics Netherlands [5]. Both household income and highest followed educational level in mothers and their partners in the study cohort suggest a selection towards a higher socio-economic status. However, differences between the population and cohort characteristics may also be due to selective missing values of ethnicity and socio-economic status in the questionnaires. Additional efforts are currently made to complete the information on ethnicity, household income and educational level in the total cohort.

Children at birth

Characteristics of the live born children at birth presented in Table 7. Among these live births, 51% were male and 49% female. These percentages are similar to the population figures in the Netherlands and in Rotterdam [6]. Ethnicity of the children was defined using the strategy as described for the mothers and partners. The differences in ethnic distributions between all newborns in the study area and the newborns participating in the study are similar to the differences found in the mothers.

Table 7. Characteristics of newborns

Male (%)	51
Birth weight (grams)*	3412 (561)
Gestational age (weeks)**	40 (35.4-42.1)
Ethnicity (%)	
Dutch, other-European	62
Surinamese	8
Moroccan	7
Turkish	8
Dutch Antilles	4
Cape Verdian	3
Other	8

Values are percentages.
*Mean (standard deviation)
** Median (95% range)
Data are based on living born children.

Overall response

Estimation of the precise number of eligible pregnant women in the study area is difficult since there is no satisfactory registry of pregnancies. Therefore, it was not attempted to identify overall response rates of pregnant women. The children form a prenatally recruited birth-cohort, thus the overall response of the study can be calculated at birth. Full participant recruitment in the definite study area was established for mothers with a delivery date from January 2003 until January 2006. The overall response in this period represents the number of children born to mothers living in the study area at their delivery date and participating in the study as percentage of the total number of children born to mothers who fulfill these eligibility criteria (Table 8). Since data collection and data cleaning is still ongoing, the response rate calculation was based on children born from January 2003 until December 2004. The number of live born children in the study area in this period is 8,494 [2]. A total of 5,189 living born children born in this period and their mothers participated in the study which gives a response rate of 61% study at birth. Children born in 2002 do not contribute to this response rate since the recruitment process did not cover the whole definite study area. Data of children born in 2005 are not expected to materially change this response rate since the enrolment rate was similar as previous years.

Table 8. Inclusion criteria at birth

Mother resident in study area at her delivery date
Date of birth of the child between April 2002 and January 2006
Informed consent by one of the parents

Postnatal follow-up

Of all 9,778 mothers, 1,195 mothers were participants in the pilot phase since they lived outside the definite study area at their delivery date. Their children are not approached for postnatal follow-up studies. Of the remaining 8,583 mothers, it is expected that 98% (n = 8,411) have pregnancies resulting in living born children that could be approached for postnatal follow-up studies (Figure 2). These mothers and their children are eligible for postnatal follow-up studies. Based on figures from 2003 and 2004, it is estimated that about 92% of these eligible mothers are willing to continue to participate in the postnatal phase with their children.

Data collection in the prenatal phase

Physical examinations

Physical examinations were planned at each visit in early pregnancy, mid-pregnancy and late pregnancy and included height, weight and blood pressure of both parents.

Overall response rates for these specific measurements in mothers and their partners are similar as the visit percentages presented in Figure 2. Since there was a wide range of gestational age at each visit, the physical examinations will be used in the analyses as gestational age adjusted measurements in early pregnancy, mid-pregnancy and late pregnancy.

Questionnaires

Mothers received four postal questionnaires and their partner received one postal questionnaire in the prenatal phase (Table 4). Each questionnaire comprises about 25 pages. Topics in these questionnaires were:
– Mother 1: medical history, family history, previous and current pregnancies, quality of life, life style habits, housing conditions, ethnicity, educational level;
– Mother 2: diet, including macronutrients and micronutrients;
– Mother 3: current pregnancy, quality of life, life style habits, psychopathology;
– Mother 4: current pregnancy, quality of life, life style habits, working conditions, household income, self-esteem;
– Partner: medical history, family history, life style habits, educational level, psychopathology.

Overall response rates for these questionnaires varied from 77% to 91% (Figure 2). However, the response rates of specific questions may be lower due to missing values within questionnaires. Because of a variety of logistic reasons, some mothers did not receive one or more questionnaires. Additional efforts are currently made to complete the information on the time independent population descriptives (medical history, ethnicity, educational level, working conditions and household income).

Fetal ultrasound examinations

Fetal ultrasound examinations were performed at each prenatal visit. Overall response rates for these ultrasound examinations were in general similar to the visit percentages given in Figure 2. These ultrasound examinations were used for both establishing gestational age and assessing fetal growth patterns. Establishing gestational age by using the first day of the last menstrual period is not reliable for a variety of reasons including the large number of women who do not know their exact date, have irregular menstrual cycles or amenorrhea, use oral contraceptive pills or bleed in early pregnancy [7]. Establishing gestational age with fetal ultrasound examinations seems to overcome most of these problems. The major disadvantage of using measurements of the ultrasound examinations for establishing gestational age is that it does not allow growth studies of these measurements since no growth variability between subjects is assumed [8]. Pregnancy dating-curves were derived in a sub-sample of the cohort including subjects with complete data on both the first day of the last menstrual period and crown-rump

length or biparietal diameter. Subsequently, gestational age at prenatal enrolment and, as a consequence, at delivery was retrospectively established by crown-rump length or biparietal diameter measured in early pregnancy or mid-pregnancy. Subsequently, longitudinal curves of all fetal growth measurements (head circumference, biparietal diameter, abdominal circumference and femur length) were created resulting in standard deviation scores for all of these specific growth measurements. Methods to examine fetal growth patterns will be developed especially focused on fetal growth retardation in different periods of pregnancy and on identifying differences between groups. These dating and growth curves and methods to examine fetal growth patterns will be published separately.

Blood and urine samples

The planned amounts of venous blood taken were 35 and 20 ml in early pregnancy and mid-pregnancy, respectively, from the mother and 10 ml from her partner in the prenatal phase. Plasma and serum samples were distributed in small aliquots (each 250 µl) and are currently stored at –80ºC. These blood samples are used for DNA extraction and stored for future studies relating genetic variants to various phenotypes and for future measurements of a variety of biomarkers for exposures and phenotypes themselves. DNA is extracted from whole blood samples. Among the participating mothers and partners, response rates for blood samples were 97% (from all visits) and 84%, respectively (Figure 2). Missing values of blood samples are mainly caused by the lack of consent. DNA extraction in children is carried out from cord blood samples and will postnatally be completed by saliva samples. Urine samples of mothers have been collected from February 2004 until November 2005 and are stored for future measurements.

Pregnancy complications and outcomes

The obstetric records of all mothers are looked up in the hospitals and midwifery practices. Specialists in the relevant field code items in these records. The major pregnancy outcomes, including live births, induced abortion and fetal or perinatal loss, are known in 99% of all enrolled mothers. In all children known to be born alive, information about gender, birth weight and gestational age is available.

Data collection in the postnatal phase

Postnatal assessments from birth until the age of 4 years are currently conducted and include:
– Physical examinations: length (height), weight, head circumference, neurological development and in the focus cohort blood pressure and body composition;

- Questionnaires: diet, behaviour, cognition, living conditions, drug use, diseases, use of medical services;
- Routine health care: screening assessments performed in the routine child health centers;
- Ultrasound examinations: in the focus cohort brain, heart and kidney development;
- Biological samples: in the focus cohort blood and saliva samples.

The details and time scheme of these measurements have been described in detail previously [1]. Current plans for measurements from the age of 4 years are focused on regular hands-on assessments in 2 to 3 hours sessions in the whole cohort and on postal questionnaires for the children and their parents.

Data management and statistical power

Data preparation

Data collected by measurements at the research centers are directly entered onto written forms and into the electronic database. Data collected by questionnaires are scanned and manually entered into an electronic database by a commercial bureau. Random samples of all questionnaires are double checked by study staff members to monitor the quality of this manual data entry process. The percentage of mistakes is kept as low as possible and does not exceed 3% per questionnaire. Open text fields are entered into the electronic database exactly as they are filled in on the questionnaires. In a secondary stage, these open text fields are cleaned and coded by a specialist in the relevant field. All measurements are centrally checked by examination of the data including their ranges, distributions, means, standard deviations, outliers and logical errors. Data outliers and missing values are checked on the original forms. The data of one specific measurement are only distributed for analyses after data collection and preparation is completed for that measurement for the whole cohort.

Privacy protection

Datasets needed for answering specific research questions are centrally built from different databases. All information in these datasets that enables identification of a particular participant (including identification number used for the logistics of the study, names and dates) is excluded before distribution to the researchers. The datasets for researchers include subject unique identification numbers that enable feedback about one subject to the data manager but do not enable identification of that particular subject.

Statistical power

Due to expected missing values and loss to follow-up, it is unlikely that most future analyses in the study will be based on data in all subjects. Therefore, power calculations demonstrated in Tables 9 and 10 are based on 7,000 subjects in the whole cohort and 700 subjects in the focus cohort. Table 9 demonstrates that for a normally distributed continuous outcome it is possible to detect with a type I error of 5% and a type II error of 20% (power 80%) a difference of 0.11 SD in the whole cohort and of 0.35 SD in the focus cohort if 10% of all subjects has the relevant exposure. Table 10 presents for dichotomous outcomes that with the same type I and II errors, it is possible to detect a relative risk of 1.39 in the whole cohort and of 2.48 in the focus cohort if 10% of all subjects has the relevant exposure and the 1 year incidence of the outcome of interest is 10%. Rates of most dichotomous environmental and genetic exposures in the Generation R Study are expected to vary generally between 10 and 20%. The presented power calculations are rather conservative since most studies will assess the effects of continuously instead of dichotomous measured exposures and studies may be focused on outcomes collected in more than only one year. Furthermore, the Generation R Study has a large number of measurements repeated over time, which may increase the accuracy of measuring the true underlying value and may thereby increase the statistical power for these measurements.

Table 9. Effects sizes in standard deviation that minimally can be detected according to the prevalence of the exposure

Proportion exposed	Whole cohort (n = 7,000)	Focus cohort (n = 700)
50%	0.067	0.212
25%	0.077	0.276
10%	0.112	0.353
5%	0.154	0.486
1%	0.337	1.064

The presented effect sizes are detectable proportions of the standard deviation with a type I error of 5% and a type II error of 20% (power 80%).

Strengths and limitations

Strengths

The design of the Generation R Study is optimal for identification of determinants in the earliest phase of life, unbiased data collection and controlling for potential confounders.

Results from existing cohort studies in children suggest the importance of early life for various health conditions in later life and have initiated several birth-cohort studies

[9-11]. Compared to other planned or recently started prospective birth-cohort studies, the size of the Generation R Study cohort is not larger but the measurements are more detailed [12-16]. The cohort is large enough to detect small effects of early environmental and genetic determinants on a variety of outcomes (Table 9-10). The detailed longitudinal fetal growth examinations enable studies into both various environmental and genetic determinants and into postnatal consequences of fetal growth and development patterns. Currently, plans are being made to carry out similar detailed regular measurements in children in the whole cohort from the age of 4 years onwards.

The study cohort is rather unique since it comprises contemporary urban children including about 50% from ethnic minorities. The largest ethnic minority groups in this population are the Surinamese, Turkish, Moroccan, Dutch Antillean and Cape Verdian groups. Knowledge about the growth, development and health of these groups is lacking. Other birth-cohort studies do not provide information about these ethnic groups. The Generation R Study enables studies into determinants of ethnic specific health problems and health care utilization habits and may contribute to the development of ethnic specific strategies for pregnant women and children.

Table 10. Relative risks that minimally can be detected according to the prevalence of the exposure

| | Incidence (1 year) of outcome of interest | | | | | |
| | Whole cohort (n = 7,000) | | | Focus cohort (n = 700) | | |
Proportion exposed	**10%**	**5%**	**1%**	**10%**	**5%**	**1%**
50%	1.23	1.33	1.83	1.83	2.28	4.94
25%	1.26	1.38	1.94	1.96	2.46	5.41
10%	1.39	1.56	2.42	2.48	3.26	7.92
5%	1.55	1.80	3.09	3.20	4.39	11.74
1%	2.36	3.04	6.83	7.75	11.61	37.55

The presented effect sizes are detectable relative risks with a type I error of 5% and a type II error of 20% (power 80%).

Limitations

Of all eligible children at birth, 61% participate in the study. National and regional registries do not have subject characteristics in all eligible children and their parents that enables detailed non-response analyses. However, the percentages of mothers from ethnic minorities and lower socio-economic status and the percentages of mothers or children with medical complications are lower among the participants than expected from the population figures in Rotterdam [2]. This selection towards a more affluent and healthy study population may be related to some determinants and outcomes separately, affecting the frequency rates and, as a consequence, the statistical power and generalizibility of the results. The prevalence and incidence rates found in the study should therefore carefully be interpreted considering the role of potential selection mechanisms. This selection leads only to bias in etiological association studies if the

selection mechanisms are related to both the determinant and outcome. Although we do not expect that this is generally the case, the potential for selection bias should be considered in each analysis. Since the main aim of the study is to examine etiological associations of early determinants with outcomes in later life instead of disease frequency rates per se, major efforts are made to keep the follow-up rates as high as possible and to prevent selective loss to follow-up.

Collaboration

The Generation R Study is conducted by several research groups from the Erasmus Medical Center Rotterdam in close collaboration with the Erasmus University Rotterdam and the Municipal Health Service Rotterdam area. Since the data collection is still ongoing and growing, the number of collaborating research groups in and outside the Netherlands is expected to increase in the coming years. The study has an open policy in regard to collaboration with other research groups. Request for collaboration should primarily be pointed to Albert Hofman (a.hofman@erasmusmc.nl). These requests are discussed in the Generation R Study Management Team regarding their study aims, overlap with ongoing studies, logistic consequences and financial contributions. After approval of the project by the Generation R Study Management Team and the Medical Ethical Committee of the Erasmus Medical Center, the collaborative research project is embedded in one of the four research areas supervised by the specific principal investigator.

References

1. Hofman A, Jaddoe VW, Mackenbach JP, Moll HA, Snijders RF, Steegers EA, et al. Growth, development and health from early fetal life until young adulthood: the Generation R Study. Paediatr Perinat Epidemiol 2004;18:61-72.
2. Center for Research and Statistics, Rotterdam (COS); http:// www.cos.rotterdam.nl; 2005.
3. Burgmeijer RJF, Merkx JAM. Pakket...En hoe pakt het uit: Ouder en Kindzorg tussen wetenschap en praktijk. Nederlands Congres Ouder en Kindzorg. Assen, the Netherlands: van Gorcum; 1999.
4. Statistics Netherlands. Allochtonen in Nederland 2004. Voorburg/Heerlen; 2004.
5. Statistics Netherlands. Standaard onderwijsindeling 2003. Voorburg/Heerlen; 2004.
6. Statistics Netherlands; www.cbs.nl. 2005.
7. Tunon K, Eik-Nes SH, Grottum P. A comparison between ultrasound and a reliable last menstrual period as predictors of the day of delivery in 15,000 examinations. Ultrasound Obstet Gynecol 1996;8:178-85.
8. Morin I, Morin L, Zhang X, Platt RW, Blondel B, Breart G, et al. Determinants and consequences of discrepancies in menstrual and ultrasonographic gestational age estimates. BJOG 2005;112:145-52.
9. Silva PA. The Dunedin multidisciplinary health and development study: a 15 year longitudinal study. Paediatr Perinat Epidemiol 1990;4:76-107.

10. Golding J, Pembrey M, Jones R. ALSPAC: the Avon Longitudinal Study of Parents and Children. I. Study methodology. Paediatr Perinat Epidemiol 2001;15:74-87.

11. Berenson GS. Bogalusa Heart Study: a long-term community study of a rural biracial (Black/White) population. Am J Med Sci 2001;322:293-300.

12. Olsen J, Melbye M, Olsen SF, Sorensen TI, Aaby P, Andersen AM, et al. The Danish National Birth Cohort: its background, structure and aim. Scand J Public Health 2001;29:300-7.

13. Smith K, Joshi H. The Millennium Cohort Study. Popul Trends 2002:107:30-4.

14. Branum AM, Collman GW, Correa A, Keim SA, Kessel W, Kimmel CA, et al. The National Children's Study of environmental effects on child health and development. Environ Health Perspect 2003;111:642-6.

15. The Norwegian mother and child cohort study; http://www.fhi.no; 2005.

16. Inskip HM, Godfrey KM, Robinson SM, Law CM, Barker DJ, Cooper C. Cohort profile: The Southampton Women's Survey. Int J Epidemiol 2006;35:42-8.

Determinants of fetal growth and birth weight

4

Active and passive maternal smoking in pregnancy and the risk of low birth weight and preterm birth

4.1

Abstract

Background: Maternal smoking in pregnancy is associated with low birth weight. The aims of this study were to examine the associations of active and passive smoking in different periods of pregnancy with low birth weight and preterm birth and to examine the effects of changing smoking habits.

Methods: This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until adulthood. Active and passive smoking were assessed by questionnaires in early pregnancy, mid-pregnancy and late pregnancy. The present analysis was based on 7,098 subjects.

Results: Active smoking until pregnancy was known, was not associated with low birth weight and preterm birth. Continued active smoking after pregnancy was known, was associated with low birth weight (adjusted odds ratio 1.64 (95% confidence interval: 1.24, 2.16)) and preterm birth (adjusted odds ratio 1.36 (95% confidence interval: 1.04, 1.78)). The strongest associations were found for active maternal smoking in late pregnancy. Passive maternal smoking in late pregnancy was only weakly associated with a continuously measured birth weight (difference -38 (95% confidence interval: -74, -3) grams between never or occasional and daily passive smoking). For all active smoking categories in early pregnancy, quitting to smoke was associated with a higher birth weight than continuing to smoke.

Conclusion: Active and passive smoking in late pregnancy are associated with adverse effects on weight and gestational age at birth. Health care strategies for pregnant women should be aimed at quitting to smoke completely rather than reducing the number of cigarettes.

Introduction

Low birth weight and preterm birth are the most important risk factors for perinatal morbidity and mortality and seem to increase the risk of developing cardiovascular disease in adulthood [1, 2]. Active maternal smoking in pregnancy is the most important modifiable risk factor for low birth weight and preterm birth in Western countries [3, 4]. Birth weight in the offspring of smoking mothers is 150 to 250 grams lower than in the offspring of non-smoking mothers [3, 4]. Although a dose-response relationship between the number of cigarettes smoked per day and birth weight has been described, the detrimental effect of smoking seems already to occur in the lowest levels of active maternal smoking [5]. The effect of changing smoking habits in pregnancy on birth weight is not well known. Previous studies suggested that quitting maternal smoking after the first trimester leads to a normal birth weight, but were not conclusive [6, 7]. The effect of reducing the number of cigarettes smoked in pregnancy on birth weight is not well known [8, 9].

More recently, it has been suggested that passive maternal smoking in pregnancy is also associated with low birth weight [10]. Studies suggested that birth weight is 25 to 75 grams lower in the offspring of mothers exposed to environmental smoke in pregnancy than in the offspring of mothers not exposed [10, 11]. To our knowledge, no studies examined the associations of passive smoking in different periods of pregnancy and of reducing the number of hours exposed to environmental smoke in pregnancy with birth weight.

In a population-based cohort study among pregnant women and their children, we examined the associations of active and passive smoking in different periods of pregnancy with low birth weight and preterm birth in the offspring. We also examined whether changing smoking habits from early to late pregnancy was associated with beneficial effects on birth weight in the offspring.

Methods

Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood. This study is designed to identify early environmental and genetic determinants of growth, development and health in fetal life, childhood and adulthood and has been described previously in detail [12, 13]. Briefly, the cohort includes 9,778 mothers and their children of different ethnicities living in Rotterdam, the Netherlands. Enrolment was aimed in early pregnancy (gestational age < 18 weeks) but was possible until birth of the child. Mothers were informed about

the study by routine health care workers (midwives, obstetricians) in pregnancy and were enrolled in the study at their routine ultrasound examination in pregnancy.

Mothers who were missed in pregnancy, were approached and enrolled in the first month after birth of their child at the routine child health centers. Assessments in pregnancy, including physical examinations, fetal ultrasounds and questionnaires, were planned in early pregnancy (gestational age < 18 weeks), mid-pregnancy (gestational age 18-25 weeks) and late pregnancy (gestational age ≥ 25 weeks). Mothers enrolled in early pregnancy (69%) had three assessments planned (in early, mid- and late pregnancy) whereas those enrolled in mid-pregnancy (19%) had two assessments (in mid- and late pregnancy) and those enrolled in late pregnancy (3%) had one assessment (in late pregnancy) planned. The individual time scheme of these assessments depended on the specific gestational age at enrolment [13]. Mothers enrolled after birth of their offspring (9%) had no prenatal assessments [12, 13]. All children were born between April 2002 and January 2006 and form a prenatally enrolled birth-cohort that is currently followed until young adulthood. Of all eligible children in the study area, 61% participated at birth in the study [13]. The study has been approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all participants.

Active and passive maternal smoking

Information about active and passive maternal smoking was obtained by postal questionnaires in early, mid- and late pregnancy [13]. Active maternal smoking at enrolment was assessed in the first questionnaire by asking whether mother smoked in pregnancy (no, until pregnancy was known, continued after pregnancy was known). This questionnaire was sent to all mothers, independent of their gestational age at enrolment. In the second and third questionnaire, the mothers were asked whether they smoked in the past 2 months (no, yes) in mid- and late pregnancy, respectively. Mothers who reported in the first questionnaire to have smoked until pregnancy was known (n = 861) but still reported to smoke in the second or third questionnaire (n = 270), were reclassified into the 'continued smoking after pregnancy was known' category. The same strategy was used for mothers who reported not to smoke in the first questionnaire (n = 5,372) but smoked in the second or third questionnaire (n = 83). Among the smoking mothers, the number of cigarettes was assessed in the following six categories: less than one per day; one to two per day; three to four per day; five to nine per day; ten to nineteen per day; and twenty or more per day. To increase the number of subjects, these categories were combined and reclassified into previously used categories: 1) non-smoking; 2) less than five cigarettes per day; 3) five to nine cigarettes per day; and 4) ten or more cigarettes per day [11]. Passive maternal smoking was assessed in each questionnaire as the number of hours per day that mothers were exposed to environmental smoke at home and at work.

For both exposure to smoke at home and at work, the following categories were used: 1) never or occasionally; 2) less than one hour per day; 3) one to three hours per day; and 4) more than three hours per day. Per questionnaire, all subjects were reclassified into the highest reported category of the exposure either at home or at work.

Covariates

Information about educational level, ethnicity and parity was obtained by the first questionnaire at enrolment in the study. Maternal alcohol consumption was assessed in early, mid- and late pregnancy by questionnaire. Maternal anthropometrics, including height and weight, were measured without shoes and heavy clothing and body mass index was calculated (weight/height^2 (kg/m^2)) in early, mid- and late pregnancy during visits at the research center.

Birth outcomes

Date of birth, birth weight and gender were obtained from midwife and hospital registries. Gestational age was established by fetal ultrasound examination because using last menstrual period has several limitations, including the large number of women who do not know their exact last menstrual period date or have irregular cycles [14].

Population for analysis

Of the total of 9,778 mothers, 91% (n = 8,880) was enrolled in pregnancy [13]. Those without information about smoking in pregnancy in the first questionnaire were excluded from the analyses (14%, n = 1,249). Of the remaining 7,631 mothers, those with twin pregnancies (n = 81), fetal deaths (n = 100) or missing birth outcomes (n = 352) were excluded. Categories of active smoking habits were similarly distributed at baseline among those with singleton live birth as outcome and those lost to follow-up. The associations of maternal smoking habits in pregnancy with birth outcomes were analyzed in the remaining 7,098 mothers. Of these mothers, 4% (n = 290) were second or third pregnancies in the study. Since there were no differences in results after exclusion of these subjects, they were included in the analyses presented. Analyses focused on smoking categories in different periods of pregnancy were restricted to mothers enrolled in early pregnancy to minimize misclassification of smoking in pregnancy period. Since both active and passive smoking were strongly correlated between mid- and late pregnancy (Spearman correlation coefficient r = 0.8, p < 0.01 for active smoking, r = 0.7, p < 0.01 for passive smoking), we did not have enough mothers to assess the separate effects of smoking in mid- and late pregnancy. Therefore, analyses were focused on early and late pregnancy. Of all mothers enrolled in early pregnancy (n = 5,502), the associations of active smoking in early pregnancy (until pregnancy was known) with birth outcomes, were assessed in those in whom information about the number of actively smoked

cigarettes was available and did not actively smoke after their pregnancy was known (n = 4,541) to exclude confounding by continued smoking. Among mothers enrolled in early pregnancy, information about the number of actively smoked cigarettes in late pregnancy was available in 83% (n = 4,580). The analyses with passive smoking in early pregnancy as determinant were conducted among non-actively smoking mothers with information about the hours of passive smoking in early pregnancy and who were never or occasionally exposed to passive smoking in mid- or late pregnancy (n = 3,334). In late pregnancy, the associations of passive smoking with birth outcomes were also analyzed among non-actively smoking mothers with information about passive smoking (n = 3,862).

Data analysis

First, the associations of active maternal smoking habits at any time during pregnancy with low birth weight and preterm birth were assessed using multiple logistic regression models. Low birth weight was defined as birth weight below the 10th percentile in the study cohort (2850 grams) and was based on infants born at a gestational age of 37 weeks or more. Preterm born children were excluded from the models with low birth weight as dependent variable. Preterm birth was defined as a gestational age of less than 37 weeks at delivery. These logistic regression models were adjusted for life style and socio-economic status related variables at enrolment (maternal body mass index, alcohol consumption, educational level) and additionally for known determinants of low birth weight and preterm birth (maternal age, height, ethnicity and parity and infant gender). Second, the associations of active and passive smoking categories in early and late pregnancy with continuously measured birth weight, low birth weight and preterm birth were assessed using multiple linear and logistic regression models. These models were adjusted for the same potential confounders. In these models, alcohol consumption and body mass index were measured in the same period of pregnancy as maternal smoking. All models with low birth weight and continuously measured birth weight as dependent variable were additionally adjusted for gestational age. Third, the associations of patterns of change in active and passive smoking categories from early to late pregnancy with birth weight were analyzed within strata of active and passive smokers in early pregnancy. Within these strata, mothers with the same active or passive smoking category in early and late pregnancy were the reference group. All measures of association are presented with their 95% confidence intervals (CI). All statistical analyses were performed using the Statistical Package of Social Sciences version 11.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Subject characteristics

Characteristics of the mothers per smoking category are presented in Table 1. Of all mothers, 25.5% (n = 1,809) reported to actively smoke in early pregnancy and 17.2% (n = 1,218) continued smoking after pregnancy was known. The age in the whole cohort ranged from 15.3 to 46.3 years with a mean of 29.8 years and was lowest in mothers who

Table 1. Maternal characteristics

	Smoking in pregnancy (n = 7,098)		
	Non-smoking	Until pregnancy was known	Continued after pregnancy was known
	n = 5,289	n = 591	n = 1,218
Age (years)	30.1 (5.1)	29.4 (5.2)	28.3 (5.8)
Height (cm)	167.4 (7.5)	168.3 (7.0)	167.1 (7.0)
Weight (kg)	69.3 (13.2)	69.4 (12.8)	70.2 (14.0)
Body mass index (kg/m^2)	24.7 (4.5)	24.5 (4.5)	25.2 (4.8)
Parity ≥ 1 (%)	45.2	31.7	45.7
Alcohol consumption (%)			
No	60.1	29.1	54.5
Until pregnancy was known	24.2	50.6	27.9
Continued	15.8	20.3	17.6
Education (%)			
Primary school	10.6	12.2	21.0
Secondary school	41.6	44.9	60.5
Higher education	47.8	42.9	18.6
Ethnicity (%)			
Dutch, other-European	57.6	62.9	57.1
Surinamese	8.4	12.4	10.4
Turkish	7.7	7.5	16.2
Moroccan	8.1	1.2	1.9
Cape Verdian	3.7	4.6	4.8
Antillean	3.4	2.9	3.8
Others	11.0	8.5	5.8
1st Child of the same mother in study (%)	95.8	95.2	96.6
Enrolment in study in early pregnancy (%)	76.4	85.8	78.2
Birth outcomes			
Birth weight (grams)	3454 (552)	3441 (559)	3251 (545)
Gestational age (weeks)*	40.0 (37.0 – 42.0)	40.0 (36.9 – 42.0)	39.9 (36.1 – 42.0)

Values are means (standard deviation) or percentages. * Median (90% range). Data were missing on height (n = 11), weight (n = 27), body mass index(n = 38), parity (n = 409), alcohol consumption (n = 19), educational level (n = 218) and ethnicity (n =60).

continued smoking after pregnancy was known. Also the percentage of mothers with higher educational level was lowest in this group. In the whole cohort, the largest ethnic groups were the Dutch and other-European (58.0%), Surinamese (9.1%), Turkish (9.1%) and Moroccan women (6.5%). Table 1 demonstrates that the percentages of Turkish mothers and Moroccan mothers were highest (16.2%) and lowest (1.9%), respectively, among those who continued smoking after pregnancy was known. Mean offspring birth weight was 3454 (SD 552) grams for mothers who did not smoke in pregnancy and 3251 (SD 545) grams for mothers who continued smoking after pregnancy was known. Of all children, 5.7% was born before a gestational age of 37 weeks.

For the 5,502 mothers who were enrolled in early pregnancy, the distributions of active and passive smoking categories in early pregnancy for various subject characteristics are presented in Table 2. The youngest age group showed the highest percentages of active and passive smoking. Among mothers using alcohol consumptions in early pregnancy, 31.0% actively smoked, whereas this percentage was 20.6% among mothers who did not use alcohol consumptions. Mothers with the highest educational level showed the lowest percentages of active and passive smoking. Both the percentages of active and passive smoking varied between the ethnic groups and were highest among Turkish women.

Table 2. Distribution of active and passive maternal smoking categories for subject characteristics in early pregnancy (n = 5,502)

	Active smoking (n = 5,479)				Passive smoking (n = 5,349)			
	Non-smoking	< 5 cigarettes per day	5 - 9 cigarettes per day	> 9 cigarettes per day [a]	Never / occasionally	< 1 hour per day	1 - 3 hours per day	> 3 hours per day
	(74.5%)	(13.5%)	(6.9%)	(5.2%)	(71.3%)	(10.2%)	(7.0%)	(11.4%)
Age (years) (n = 5,502)								
< 28 years (33.4%)	66.9	18.2	8.5	6.3	59.9	13.0	9.7	17.4
28-32 years (30.9%)	78.3	11.8	5.7	4.3	75.2	10.3	5.4	9.1
> 32 years (35.8%)	78.2	10.6	6.4	4.8	78.4	7.6	6.1	7.8
Body mass index (kg/m²) (n = 5,476)								
< 22 kg/m² (31.8%)	75.0	14.8	6.0	4.1	74.1	9.2	6.1	10.6
22-25 kg/m² (33.3%)	75.3	13.7	6.5	4.5	72.9	10.5	6.7	9.8
> 25 kg/m² (34.8%)	73.1	12.0	8.2	6.7	67.2	10.9	8.2	13.8
Alcohol consumption (n = 5,488)								
No (53.5%)	79.3	10.7	5.8	4.1	68.1	11.7	7.8	12.5
Yes (46.5%)	69.1	16.5	8.1	6.4	71.9	10.7	6.3	11.2
Education (n = 5,355)								
Primary school (10.9%)	62.1	16.5	11.1	10.3	58.0	12.1	9.4	20.5
Secondary school (44.2%)	68.6	15.9	9.4	6.2	61.5	12.5	9.3	16.7
Higher education (44.9%)	83.5	10.1	3.5	2.9	84.0	7.6	4.2	4.1

Table 2 (continued).

	Active smoking (n = 5,479)				Passive smoking (n = 5,349)			
	Non-smoking	< 5 cigarettes per day	5 - 9 cigarettes per day	> 9 cigarettes per day [a]	Never / occasionally	< 1 hour per day	1 - 3 hours per day	> 3 hours per day
	(74.5%)	(13.5%)	(6.9%)	(5.2%)	(71.3%)	(10.2%)	(7.0%)	(11.4%)
Parity (n = 5,201)								
0 (57.7%)	73.6	14.3	6.9	5.2	68.9	11.6	7.0	12.6
≥ 1 (42.3%)	76.2	11.7	7.4	4.6	75.2	8.8	6.9	9.1
Ethnicity (n = 5,457)								
Dutch / European (61.6%)	74.5	12.2	7.5	5.9	73.8	9.4	6.5	10.3
Surinamese (8.6%)	69.3	20.4	5.6	4.7	63.8	13.0	7.9	15.2
Turkish (8.2%)	62.4	20.6	11.4	5.6	55.9	13.2	12.3	18.5
Moroccan (5.7%)	94.2	3.5	1.9	0.3	75.1	11.7	5.1	8.1
Cape Verdian (3.8%)	68.3	16.1	8.3	7.3	66.5	13.4	6.7	13.4
Antillean (2.9%)	72.2	19.0	5.7	3.2	62.6	13.5	7.1	16.8
Others (9.2%)	82.1	11.4	3.2	3.2	78.1	6.8	6.4	8.7

Values are percentages.
[a] Within this category, 79% of the subjects smoked 10 to 19 cigarettes per day

Active smoking habits during pregnancy

Table 3 presents the adjusted odds ratios (aOR) for the associations of active maternal smoking in pregnancy with low birth weight and preterm birth. Smoking until pregnancy was known, was not associated with an increased risk of low birth weight and preterm birth. In all models, continued smoking after pregnancy was known, was associated with low birth weight (aOR 1.64 (95% CI: 1.24, 2.16)) and preterm birth (aOR 1.36 (95% CI: 1.04, 1.78)).

Active and passive maternal smoking categories in early and late pregnancy

Table 4 and 5 present the associations of active and passive smoking categories in early and late pregnancy with continuously measured birth weight, low birth weight and preterm birth. Active smoking categories until pregnancy was known, were not associated with low birth weight and preterm birth (Table 4). Active maternal smoking in late pregnancy was strongly associated with continuously measured birth weight. The largest effect estimate was seen for smoking five to nine cigarettes per day (difference in birth weight -200 (95% CI: -277, -122) grams). The risk of low birth weight was increased in mothers who smoked five to nine cigarettes per day (aOR 1.83 (95% CI: 1.07, 3.13)) and who smoked more than nine cigarettes per day (aOR 2.08 (95% CI: 1.00, 4.34)). We found only an increased risk of preterm birth for smoking more than nine cigarettes per day (aOR 2.52 (95% CI: 1.36, 4.67)). Among non-actively smoking mothers, passive smoking in early and late pregnancy was not associated with continuously measured

Table 3. Odds ratios for the associations of active smoking habits in pregnancy with low birth weight and preterm birth

	Odds ratio for low birth weight		
Active smoking in pregnancy n = 6,692 [a]	**Model A**	**Model B**	**Model C**
Non-smoking n = 5,007	*Reference*	*Reference*	*Reference*
Until pregnancy was known n = 558	1.16 (0.81, 1.65)	1.15 (0.80, 1.67)	1.03 (0.68, 1.55)
Continued after pregnancy was known n = 1,127	1.73 (1.37, 2.19)**	1.57 (1.22, 2.02)**	1.64 (1.24, 2.16)**
	Odds ratio for preterm birth		
Active smoking in pregnancy n = 7,098	**Model A**	**Model B**	**Model C**
Non-smoking n = 5,289	*Reference*	*Reference*	*Reference*
Until pregnancy was known n = 591	1.05 (0.73, 1.52)	1.11 (0.76, 1.62)	0.94 (0.63, 1.40)
Continued after pregnancy was known n = 1,218	1.43 (1.12, 1.83)**	1.36 (1.04, 1.76)*	1.36 (1.04, 1.78)*

Values are odds ratios (95% confidence interval).
Model A: unadjusted.
Model B: adjusted for maternal body mass index, alcohol consumption, educational level.
Model C: adjusted for maternal body mass index, alcohol consumption, educational level, height, ethnicity, parity and age and infant gender.
[a] Preterm born children excluded
 All models with low birth weight as dependent variable are additionally adjusted for gestational age.
*p-value < 0.05, **p-value < 0.01

birth weight, low birth weight and preterm birth (Table 5). However, weak tendencies for daily passive smoking in late pregnancy with continuously measured birth weight were found (difference in birth weight -29 (95% CI: -78, 19) grams for less than one hour per day, -50 (95% CI: -114, 15) grams for one to three hours per day and -44 (95% CI: -108, 20) grams for more than three hours per day). Combining these categories in two larger groups of 1) never or occasional passive smoking and 2) daily passive smoking demonstrated a difference in birth weight of -38 (95% CI: -74, -3) grams for daily passive smoking in late pregnancy compared to never or occasional passive smoking (results not shown in Table 5).

Table 4. Associations of active smoking categories in early and late pregnancy with birth outcomes

	Difference in birth weight (grams) [a]	Adjusted odds ratio for low birth weight [b, c]	Adjusted odds ratio for preterm birth [b]
Early pregnancy (until pregnancy was known, < 18 weeks)	n = 4,541	n = 4,301	n = 4,541
Non-smoking	*Reference* n = 4,022	*Reference* n = 3,811	*Reference* n = 4,022
< 5 cigarettes per day	44 (-9, 96) n = 298	0.74 (0.40, 1.38) n = 283	0.85 (0.47, 1.52) n = 298
5 - 9 cigarettes per day	-9 (-92, 73) n = 106	1.42 (0.62, 3.32) n = 102	0.68 (0.25, 1.88) n = 106
> 9 cigarettes per day	45 (-37, 128) n = 115	0.48 (0.14, 1.62) n = 105	1.48 (0.73, 3.02) n = 115
Late pregnancy (≥ 25 weeks)	n = 4,580	n = 4,345	n = 4,580
Non-smoking	*Reference* n = 3,897	*Reference* n = 3,707	*Reference* n = 3,897
< 5 cigarettes per day	-100 (-150, -49)** n = 327	1.43 (0.87, 2.36) n = 310	1.06 (0.62, 1.82) n = 327
5 - 9 cigarettes per day	-165 (-224, -107)** n = 225	1.83 (1.07, 3.13)* n = 211	1.25 (0.70, 2.23) n = 225
> 9 cigarettes per day	-200 (-277, -122)** n = 131	2.08 (1.00, 4.34) n = 117	2.52 (1.36, 4.67)** n = 131

[a] Values are regression coefficients (95% confidence interval) and number of subjects per smoking category. [b] Values are odds ratios (95% confidence interval) and number of subjects per smoking category. [c] Preterm born children excluded. All models are adjusted for maternal body mass index, alcohol consumption, educational level, ethnicity, parity, height and age and infant gender. All models with birth weight and low birth weight as dependent variable are additionally adjusted for gestational age. Active smoking models in early pregnancy are based on mothers who did not actively smoke after pregnancy was known.
*p-value < 0.05, **p-value < 0.01

Changing smoking patterns in pregnancy

Table 3 shows that mothers who quitted smoking after their pregnancy was known, did not have an increased risk of having a low birth weight infant. Table 6 presents the effect of changing smoking categories from early to late pregnancy on birth weight for different categories of active and passive smoking in early pregnancy. For all categories of active smoking in early pregnancy, a higher birth weight was found for quitting to smoke. Reducing the number of actively smoked cigarettes from five to nine per day or more than nine per day in early pregnancy to less than five per day in late pregnancy without stopping completely was associated with smaller, non-significant, changes of birth weight. We did not find associations between changing the hours of passive smoking from early to late pregnancy and birth weight.

Table 5. Associations of passive smoking categories in early and late pregnancy with birth outcomes

	Difference in birth weight (grams) [a]	Adjusted odds ratio for low birth weight [b,c]	Adjusted odds ratio for preterm birth [b]
Early pregnancy (< 18 weeks)	n = 3,334	n = 3,173	n = 3,334
Never / occasionally	*Reference* n = 2,689	*Reference* n = 2,563	*Reference* n = 2,689
< 1 hour per day	1 (-50, 53) n = 307	0.81 (0.45, 1.45) n = 290	1.03 (0.60, 1.77) n = 307
1 - 3 hours per day	-18 (-88, 52) n = 160	1.59 (0.79, 3.14) n = 154	0.79 (0.34, 1.83) n = 160
> 3 hours per day	-38 (-105, 29) n = 178	1.25 (0.61, 2.56) n = 166	0.94 (0.46, 1.91) n = 178
Late pregnancy (≥ 25 weeks)	n = 3,862	n = 3,674	n = 3,862
Never / occasionally	*Reference* n = 3,126	*Reference* n = 2,977	*Reference* n = 3,126
< 1 hour per day	-29 (-78, 19) n = 344	1.23 (0.74, 2.04) n = 327	0.90 (0.54, 1.53) n = 344
1 - 3 hours per day	-50 (-114, 15) n = 194	0.92 (0.43, 1.99) n = 182	1.04 (0.53, 2.05) n = 194
> 3 hours per day	-44 (-108, 20) n = 198	1.35 (0.71, 2.59) n = 188	0.66 (0.30, 1.44) n = 198

[a] Values are regression coefficients (95% confidence interval) and number of subjects per smoking category. [b] Values are odds ratios (95% confidence interval) and number of subjects per smoking category. [c] Preterm born children excluded. All models are adjusted for maternal body mass index, alcohol consumption, educational level, ethnicity, parity, height and age and infant gender. All models with birth weight and low birth weight as dependent variable are additionally adjusted for gestational age. Passive smoking models in early pregnancy are based on mothers who did not passively smoke in mid- or late pregnancy

Discussion

This population-based prospective cohort study showed associations of active maternal smoking in late pregnancy with low birth weight and preterm birth. Passive smoking in pregnancy was weakly associated with a lower birth weight in the offspring. Quitting active smoking after pregnancy was known, was associated with a higher birth weight in the offspring compared to continued smoking or reducing the numbers of cigarettes without quitting completely.

Methodological considerations

The strength of this study is the population-based cohort with a large number of sub-jects studied from early pregnancy. To our knowledge, this is the largest cohort study examining the associations between maternal smoking in pregnancy and birth weight

Table 6. Associations of changing active and passive smoking habits in pregnancy with birth weight

Active smoking	Late pregnancy (≥ 25 weeks)			
Early pregnancy (< 18 weeks)	Quitted [a]	< 5 cigarettes per day	5 - 9 cigarettes per day	> 9 cigarettes per day
< 5 cigarettes per day *n = 579*	144 (66, 221)** *n = 278*	*Reference* *n = 221*	-41 (-157, 73) *n = 71*	-137 (-445, 171) *n = 9*
5 - 9 cigarettes per day *n = 306*	154 (27, 281)* *n = 91*	126 (-33, 285) *n = 44*	*Reference* *n = 121*	23 (-126, 173) *n = 50*
> 9 cigarettes per day *n = 223*	187 (14, 360)* *n = 98*	109 (-125, 343) *n = 30*	-26 (-251, 198) *n = 27*	*Reference* *n = 68*
Passive smoking	Late pregnancy (≥ 25 weeks)			
Early pregnancy (< 18 weeks)	Never / occasionally	< 1 hour per day	1 - 3 hours per day	> 3 hours per day
< 1 hour per day *n = 301*	13 (-82, 109) *n =120*	*Reference* *n = 138*	20 (-112, 153) *n = 30*	-66 (-242, 110) *n =13*
1 - 3 hours per day *n = 159*	105 (-48, 258) *n = 33*	37 (-119, 192) *n = 45*	*Reference* *n = 60*	53 (-122, 228) *n = 21*
> 3 hours per day *n = 171*	12 (-123, 147) *n = 33*	25 (-140, 190) *n = 14*	49 (-58, 158) *n = 34*	*Reference* *n = 90*

Values are regression coefficients (95% CI) and reflect the difference in birth weight compared to the reference group. All models were adjusted for maternal change in maternal body mass index, height, alcohol consumption, educational level, ethnicity, parity and age and fetal gestational age and gender. [a] Includes mothers who immediately quitted active smoking after their pregnancy was known and mothers who quitted smoking between early and late pregnancy.
*p-value < 0.05, **p-value < 0.01

with data on both active and passive smoking in different trimesters of pregnancy published so far. Follow-up until birth was available for 93% and information about a large number of potential confounders was available in this study.

Information about smoking in pregnancy at enrolment was missing in 14% of all prenatally enrolled mothers. In the offspring of these mothers, birth weight was 41 (95% CI: 6, 76) grams lower. Non-response for the main determinant may be selective if the percentage of smoking mothers is higher among those without than those with information about smoking. This would probably lead to loss of power and underestimation of the estimated adverse effects of smoking on birth outcomes. Among mothers with information about their smoking habits at baseline, categories of smoking habits were similarly distributed among those lost to follow-up and those with a singleton live birth as pregnancy outcome. Lost to follow-up would lead to selection bias if the associations of maternal smoking in pregnancy with low birth weight and preterm birth differ between those lost to follow-up and those with singleton live birth as pregnancy outcome. This seems unlikely but can not be excluded.

Information about maternal smoking in pregnancy was collected by questionnaires in pregnancy without reference to the birth outcomes. Although assessing smok-

ing habits in pregnancy by questionnaires seems to be a valid method, misclassification may occur [15]. Underreporting of maternal smoking across the various smoking categories may be present. The difference in birth weight between the offspring of non-smoking and low to moderate smoking mothers may be overestimated if underreporting would be selectively present among heavy smoking mothers who report low to moderate smoking. To overcome these limitations, other studies used biomarkers of tobacco exposure including cotinine in maternal urine samples [16, 17]. However, low correlations between cotinine levels and self reported smoking habits have been demonstrated [18]. Possible explanations for these low correlations include inaccurate maternal reporting of smoking habits in pregnancy, use of categories of number of cigarettes smoked in questionnaires and individual differences in inhalation, absorption and metabolism. Previous studies demonstrated that use of cotinine levels is not superior to self-report in studying the effect of maternal smoking in pregnancy on birth weight [5, 19]. We assessed smoking as categorical and not as continuous variable since data collection and data cleaning are more easily conducted in a large cohort. Inherently, this diminishes power to identify exposure effects because information within categories is lost. Maternal passive smoking was assessed as hours exposed to environmental smoke at home and at work. This method may introduce error since it does not include exposure to smoke outside work and home.

Main outcomes in our study were low birth weight and preterm birth. Additional models with continuously measured birth weight as outcome were adjusted for gestational age since interest in these models was in fetal growth retardation. Fetal growth retardation due to maternal smoking in pregnancy does not have to lead to low birth weight if the fetus was supposed to grow on the upper percentiles. Misclassification in the outcome, as measure of fetal growth retardation, can therefore not be excluded.

Active maternal smoking in pregnancy

As in previous studies, we found strong associations between active smoking in pregnancy and low birth weight and preterm birth [3, 4, 20]. These associations were independent of potential confounders. Active smoking in late pregnancy was strongly associated with low birth weight and preterm birth. The detrimental effect was already seen in the lowest smoking category. This is in line with other studies demonstrating that low levels of maternal smoking measured by questionnaires and urine cotinine levels have already substantial effects on birth weight [15, 21]. Changing smoking habits in pregnancy seems to be most beneficial when mother quits smoking in early pregnancy. No differences in birth weight were observed between the offspring of mothers who quitted smoking after their pregnancy was known and the offspring of mothers who never smoked. Previous studies supported this finding and even described an increase in birth weight in the offspring of mothers who quitted smoking in early pregnancy [6].

This may be partly explained by an increase in maternal body mass index in pregnancy in mothers who quit smoking in pregnancy. Reducing the number of cigarettes from more than five per day to less than five per day without quitting completely, was associated with smaller beneficial effects on birth weight. However, since we had only a small number of mothers who smoked more than five cigarettes per day in early pregnancy that reduced the number of cigarettes without quitting and we used smoking as categorized variable, our study may lack power to demonstrate small effects of reducing the number of cigarettes.

Passive maternal smoking in pregnancy

Our results demonstrated weak associations of maternal passive smoking in late pregnancy with birth weight. The previously suggested effect sizes of passive maternal smoking on decrease in birth weight in the offspring ranged from 25 to 75 grams [10]. Our results are in line with these studies. However, studies in large cohorts examining the effect of passive maternal smoking on low birth weight did not measure passive smoking in various periods of pregnancy [11]. We found only a weak effect of passive smoking in late pregnancy on birth weight. Both quitting and reducing the number of hours of passive smoking were not associated with beneficial effects on birth weight in the offspring. This may be explained by the overall small effect size of passive maternal smoking and the small number of subjects in which the number of hours of passive smoking changed in our study.

Study implications

Our study showed that active maternal smoking, especially in late pregnancy, is associated with an increased risk of low birth weight and preterm birth in the offspring. Health care strategies for pregnant women should be primarily aimed at quitting smoking completely in early pregnancy rather than reducing the number of cigarettes. Passive smoking should be prevented in pregnant women. Since birth weight is only a crude measure of fetal growth, studies focused on fetal growth patterns are necessary.

References

1.   Walsh RA. Effects of maternal smoking on adverse pregnancy outcomes: examination of the criteria of causation. Hum Biol 1994;66:1059-92.
2.   Barker DJ. Fetal origins of coronary heart disease. BMJ 1995;311:171-4.
3.   Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. Bull World Health Organ 1987;65:663-737.
4.   Abel EL. Smoking during pregnancy: a review of effects on growth and development of offspring. Hum Biol 1980;52:593-625.

5. England LJ, Kendrick JS, Gargiullo PM, Zahniser SC, Hannon WH. Measures of maternal tobacco exposure and infant birth weight at term. Am J Epidemiol 2001;153:954-60.
6. MacArthur C, Knox EG. Smoking in pregnancy: effects of stopping at different stages. Br J Obstet Gynaecol 1988;95:551-5.
7. Lieberman E, Gremy I, Lang JM, Cohen AP. Low birthweight at term and the timing of fetal exposure to maternal smoking. Am J Public Health 1994;84:1127-31.
8. Li CQ, Windsor RA, Perkins L, Goldenberg RL, Lowe JB. The impact on infant birth weight and gestational age of cotinine-validated smoking reduction during pregnancy. JAMA 1993;269:1519-24.
9. Secker-Walker RH, Vacek PM, Flynn BS, Mead PB. Estimated gains in birth weight associated with reductions in smoking during pregnancy. J Reprod Med 1998;43:967-74.
10. Misra DP, Nguyen RH. Environmental tobacco smoke and low birth weight: a hazard in the workplace? Environ Health Perspect 1999;107S:897-904.
11. Windham GC, Hopkins B, Fenster L, Swan SH. Prenatal active or passive tobacco smoke exposure and the risk of preterm delivery or low birth weight. Epidemiology 2000;11:427-33.
12. Hofman A, Jaddoe VW, Mackenbach JP, Moll HA, Snijders RF, Steegers EA, et al. Growth, development and health from early fetal life until young adulthood: the Generation R Study. Paediatr Perinat Epidemiol 2004;18:61-72.
13. Jaddoe VW, Mackenbach JP, Moll HA, Steegers EA, Tiemeier H, Verhulst FC et al. The Generation R Study: study design and cohort profile. Eur J Epidemiol 2006. In press.
14. Tunon K, Eik-Nes SH, Grottum P. A comparison between ultrasound and a reliable last menstrual period as predictors of the day of delivery in 15,000 examinations. Ultrasound Obstet Gynecol 1996;8:178-85.
15. Klebanoff MA, Levine RJ, Morris CD, Hauth JC, Sibai BM, Ben Curet L, et al. Accuracy of self-reported cigarette smoking among pregnant women in the 1990s. Paediatr Perinat Epidemiol 2001;15:140-3.
16. Hebel JR, Fox NL, Sexton M. Dose-response of birth weight to various measures of maternal smoking during pregnancy. J Clin Epidemiol 1988;41:483-9.
17. Wang X, Tager IB, Van Vunakis H, Speizer FE, Hanrahan JP. Maternal smoking during pregnancy, urine cotinine concentrations, and birth outcomes. A prospective cohort study. Int J Epidemiol 1997;26:978-88.
18. English PB, Eskenazi B, Christianson RE. Black-white differences in serum cotinine levels among pregnant women and subsequent effects on infant birthweight. Am J Public Health 1994;84:1439-43.
19. Haddow JE, Knight GJ, Palomaki GE, Kloza EM, Wald NJ. Cigarette consumption and serum cotinine in relation to birthweight. Br J Obstet Gynaecol 1987;94:678-81.
20. Bernstein IM, Mongeon JA, Badger GJ, Solomon L, Heil SH, Higgins ST. Maternal smoking and its association with birth weight. Obstet Gynecol 2005;106:986-91.
21. England LJ, Kendrick JS, Wilson HG, Merritt RK, Gargiullo PM, Zahniser SC. Effects of smoking reduction during pregnancy on the birth weight of term infants. Am J Epidemiol 2001;154:694-701.

Maternal smoking in pregnancy and fetal growth characteristics

4.2

Abstract

Background: Maternal smoking in pregnancy is associated with low birth weight. Low birth weight is a crude measure of fetal growth. The aim of this study was to examine the associations of smoking in pregnancy with different fetal growth characteristics.

Methods: This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until adulthood. Smoking was assessed by questionnaires in early, mid- and late pregnancy. Fetal growth measurements used for the present study included head circumference, abdominal circumference and femur length measured in mid- and late pregnancy. The present analysis was based on 7,098 subjects.

Results: Continued maternal smoking after pregnancy was known, was associated with reduced fetal growth of head circumference (-0.56 (95% confidence interval: -0.73, -0.40) mm per week), abdominal circumference (-0.58 (95% confidence interval: -0.81, -0.34) mm per week) and femur length (-0.19 (95% confidence interval: -0.23, -0.14) mm per week). Maternal smoking until pregnancy was known, was associated with an increased abdominal circumference growth (0.35 (95% confidence interval: 0.05, 0.65) mm per week). Continued maternal smoking was associated with smaller fetal femur length from mid-pregnancy (gestational age 18 - 25 weeks) onwards and smaller head and abdominal circumference from late pregnancy (gestational age ≥ 25 weeks). Analyses with standard deviation scores for the growth characteristics, demonstrated the largest effect estimates for femur length.

Conclusion: Maternal smoking in pregnancy is associated with reduced growth of fetal head circumference, abdominal circumference and femur length. The larger effect on femur length suggests that smoking in pregnancy affects primarily peripheral tissues.

Introduction

Maternal smoking in pregnancy is the most important modifiable risk factor for low birth weight in Western countries [1, 2]. Birth weight is 150 to 250 grams lower in the offspring of mothers who smoke in pregnancy [1, 2]. Smoking in pregnancy leads to low birth weight by decreased fetal supplies of both nutrients and oxygen and subsequently fetal growth retardation. Since low birth weight is only a proxy for fetal growth retardation, it is an inappropriate measure for assessing the adverse effects of smoking in pregnancy on fetal growth and development. Fetal growth retardation may lead to normal birth weight if the fetus is actually supposed to grow on the upper percentiles based on the genetic growth potential. Detailed anthropometric measurements at birth including length, head circumference and abdominal circumference may be more informative than weight but are also the result of various fetal growth patterns.

A limited number of previous studies examined the effect of maternal smoking in pregnancy on fetal growth [3-8]. These studies suggested that maternal smoking in pregnancy is associated with impaired fetal growth from a gestational age of 20 weeks onwards. However, these studies were conducted in small groups or in hospital-based populations, were not able to adjust for all potential confounders and did not examine the effects of maternal smoking on various fetal growth characteristics in different periods of pregnancy. This may be relevant for identifying specific critical periods for the effect of maternal smoking on fetal growth.

We examined in a population-based cohort study in pregnant women the associations of maternal smoking in pregnancy with longitudinally measured fetal growth characteristics.

Methods

Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood. This study is designed to identify early environmental and genetic determinants of growth, development and health in fetal life, childhood and adulthood and has been described previously in detail [9, 10]. Briefly, the cohort includes 9,778 mothers and their children of different ethnicities living in Rotterdam, the Netherlands. Enrolment was aimed in early pregnancy (gestational age < 18 weeks) but was possible until birth of the child. Mothers were informed about the study by routine health care workers in pregnancy (midwives, obstetricians) and were enrolled in the study at their routine fetal ultrasound examination in pregnancy. Mothers who were missed in pregnancy, were approached and enrolled in the first month after birth

of their child at the routine child health centers. Assessments in pregnancy, including physical examinations, fetal ultrasound examinations and questionnaires, were planned in early pregnancy (gestational age < 18 weeks), mid-pregnancy (gestational age 18-25 weeks) and late pregnancy (gestational age ≥ 25 weeks). Mothers enrolled in early pregnancy (69%) had three assessments planned (in early, mid- and late pregnancy) whereas those enrolled in mid-pregnancy (19%) had two assessments (in mid- and late pregnancy) and those enrolled in late pregnancy (3%) had one assessment (in late pregnancy) planned. The individual time scheme of these assessments depended on the specific gestational age at enrolment [10]. Mothers enrolled after birth of their off-spring (9%) had no prenatal assessments. All children were born between April 2002 and January 2006 and form a prenatally enrolled birth-cohort that is currently followed until young adulthood. Of all eligible children in the study area, 61% participated at birth in the study [10]. The study has been approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all participants.

Maternal smoking

Information about maternal smoking was obtained by postal questionnaires in early, mid- and late pregnancy [10]. Maternal smoking at enrolment was assessed in the first questionnaire by asking whether mother smoked in pregnancy (no, until pregnancy was known, continued after pregnancy was known). This questionnaire was sent to all mothers, independent of their gestational age at enrolment. In the second and third questionnaire, the mothers were asked whether they smoked in the past 2 months (no, yes) in mid- and late pregnancy, respectively. Mothers who reported in the first question-naire to have smoked until pregnancy was known (n = 861) but still reported to smoke in the second or third questionnaire (n = 270), were reclassified into the 'continued smok-ing after pregnancy was known' category. The same strategy was used for mothers who reported not to smoke in the first questionnaire (n = 5,372) but smoked in the second or third questionnaire (n = 83). Among the smoking mothers, the number of cigarettes was assessed in the following six categories: less than one per day; one to two per day; three to four per day; five to nine per day; ten to nineteen per day; and twenty or more per day. To increase the number of subjects, these categories were combined and reclassified into the following categories: 1) non-smoking; 2) less than five cigarettes per day; 3) five to nine cigarettes per day; and 4) ten or more cigarettes per day.

Fetal ultrasound examinations

Fetal ultrasound examinations were carried out at the visits at one of the research cen-ters in early, mid- and late pregnancy. These fetal ultrasound examinations were used for both establishing gestational age and assessing fetal growth characteristics. Gestational

age was established by fetal ultrasound examination because using last menstrual period has several limitations including the large number of women who do not know their exact last menstrual period date or have irregular menstrual cycles [11]. Pregnancy dating curves were constructed on subjects in the study with complete data on gestational age measured by ultrasound and last menstrual period. Crown-rump length was used for pregnancy dating until a gestational age of 12 weeks (crown-rump length smaller than 80 mm) and biparietal diameter was used for pregnancy dating thereafter (gestational age from 12 weeks onwards, biparietal diameter larger than 20 mm) [12, 13]. Fetal growth measurements used for the present study included head circumference, abdominal circumference and femur length measured in mid- and late pregnancy. Early pregnancy was not included since these fetal ultrasound examinations were primarily performed to establish gestational age. Growth characteristics were measured to the nearest millimeter using standardized ultrasound procedures [14]. Gestational age adjusted standard deviation scores were constructed for all fetal growth measurements. These were based on reference growth curves from the whole study population. The median (95% range) gestational age for the fetal ultrasound examinations in early, mid- and late pregnancy was 13.1 (9.3 - 17.5) weeks, 20.5 (18.4 - 23.3) weeks and 30.4 (27.9 - 33.0) weeks, respectively. For mothers who had more than one ultrasound examination in one of these periods, only the ultrasound examination closest to the median of these visits was included in the present study.

Covariates

Information about educational level, ethnicity and parity was obtained by the first questionnaire at enrolment in the study. Maternal anthropometrics, including height (m) and weight (kg), were measured without shoes and heavy clothing and body mass index was calculated (weight/height^2 (kg/m^2)) in early, mid- and late pregnancy during visits at the research center.

Population for analysis

Of the total of 9,778 mothers, 91% (n = 8,880) was enrolled in pregnancy [10]. Mothers without information about smoking in pregnancy in the first questionnaire were excluded from the present study (14%, n = 1,249). Of the remaining 7,631 mothers, those with twin pregnancies (n = 81), fetal deaths (n = 100) or missing birth outcomes (n = 352) were excluded since main interest was in low risk singleton pregnancies. Categories of active smoking habits were similarly distributed at baseline among those with singleton live birth as pregnancy outcome and those lost to follow-up. The associations of maternal smoking habits during pregnancy with longitudinally measured fetal growth characteristics were analyzed in the remaining 7,098 mothers. Of these mothers, 4.1% were second (n = 284) or third (n = 6) pregnancies in the study. Since there were no

differences in results after exclusion of these subjects, they were included in the analyses presented. Analyses focused on smoking categories in mid- and late pregnancy were restricted to mothers who enrolled in the study in early pregnancy (n = 5,502) to minimize misclassification of smoking in pregnancy period. Of these mothers, information about smoking and fetal growth was available in 85% (n = 4,655) in mid-pregnancy and in 83% (n = 4,542) in late pregnancy.

Data analysis

The associations between maternal smoking habits during pregnancy and repeatedly measured growth characteristics (head circumference, abdominal circumference, femur length) were analyzed using unbalanced repeated measurement analysis. The best fitting models were constructed using fractional polynomials of gestational age [15]. Maternal smoking during pregnancy (no, until pregnancy was known, continued after pregnancy was known) was included in these models as interaction term with gestational age (p-value < 0.05). The models can be written as:

Head circumference = β_0 + β_1*smoking + β_2*gestational age^2 + β_3*gestational age^2*ln(gestational age) + β_4*smoking*gestational age.

Femur length = β_0 + β_1*smoking + β_2*gestational age + β_3*gestational age^3 + β_4*smoking*gestational age.

The model structure for abdominal circumference was similar as the model for head circumference. In these models, 'β_0 + β_1*smoking' reflects the intercept and 'β_2*gestational age^2 + β_3*gestational age^2*ln (gestational age)' for head circumference and abdominal circumference and 'β_2*gestational age + β_3*gestational age^3' for femur length reflect the slope of growth per week. The terms including 'β_4' reflects the differences in growth of each fetal characteristic between the maternal smoking categories. All models were additionally adjusted for life style and socio-economic status related confounders (maternal body mass index, educational level) and other known determinants of fetal growth (maternal age, height, ethnicity and parity and fetal gender) [1]. Using the same strategy, additional models were constructed for standard deviation scores of these growth characteristics. The best fitting model for these growth characteristics included the terms:

Standard deviation score = β_0 + β_1*smoking + β_2*gestational age + β_3*gestational age^{-2} + β_4*smoking*gestational age

This model was used for head circumference, abdominal circumference and femur length. The associations of categories of the number of cigarettes smoked per day with these standard deviation scores in mid- and late pregnancy were assessed using multiple regression models.These models were adjusted for maternal body mass index and educational level and subsequently for maternal age, height, ethnicity and parity and fetal gender. All measures of association are presented with their 95% confidence intervals (CI). The statistical analyses were performed using the Statistical Analysis System version 8.2 (SAS, Stata corporation, College station, TX, USA), including the Proc Mixed module for unbalanced repeated measurements [16].

Results

Subject characteristics

Characteristics of the mothers per smoking category are presented in Table 1. Of all mothers, 25.5% (n = 1,809) reported to actively smoke in early pregnancy and 17.2% (n = 1,218) continued smoking after pregnancy was known. The age in the whole cohort ranged from 15.3 to 46.3 years with a mean of 29.8 years and was lowest in mothers who continued smoking after pregnancy was known. Also the percentage of mothers with higher educational level was lowest in this group. In the whole cohort, the largest ethnic groups were the Dutch and other-European (58.0%), Surinamese (9.1%), Turkish (9.1%) and Moroccan women (6.5%). Table 1 demonstrates that the percentages of Turkish mothers and Moroccan mothers were highest (16.2%) and lowest (1.9%), respectively, among those who continued smoking after pregnancy was known. Mean offspring birth weight was 3454 (SD 552) grams for mothers who did not smoke in pregnancy and 3251 (SD 545) grams for mothers who continued smoking after pregnancy was known.

Maternal smoking habits during pregnancy

The associations between maternal smoking habits during pregnancy and longitudinally measured fetal growth characteristics are presented in Table 2. Compared to non-smoking, smoking until pregnancy was known was not associated with growth differences in head circumference and femur length. Growth of the fetal abdominal circumference was higher among mothers who quitted smoking after their pregnancy was known compared to non-smokers (0.35 (95% CI: 0.05, 0.65) mm per week). In the fully adjusted models, continued smoking after pregnancy was known was inversely associated with fetal growth of the head circumference (-0.56 (95% CI: -0.73, -0.40) mm per week), abdominal circumference (-0.58 (95% CI: -0.81, -0.34) mm per week) and femur length (-0.19 (95% CI: -0.23, -0.14) mm per week). Figure 1 presents the estimated differences in standard deviation scores for fetal head circumference, abdominal circumference and

femur length between mothers who did not smoke in pregnancy and mothers who continued to smoke after pregnancy was known. Differences between non-smoking and continued smoking after pregnancy was known, increased with increasing gestational age for all three fetal growth characteristics. The largest effect was seen for femur length. No differences in fetal growth characteristics, expressed as standard deviation scores, were found between non-smoking mothers and mothers who smoked until pregnancy was known (not presented in Figure 1).

Table 1. Maternal characteristics

	Smoking in pregnancy (n = 7,098)		
	Non-smoking	**Until pregnancy was known**	**Continued after pregnancy was known**
	n = 5,289	n = 591	n = 1,218
Age (years)	30.1 (5.1)	29.4 (5.2)	28.3 (5.8)
Height (cm)	167.4 (7.5)	168.3 (7.0)	167.1 (7.0)
Weight (kg)	69.3 (13.2)	69.4 (12.8)	70.2 (14.0)
Body mass index (kg/m²)	24.7 (4.5)	24.5 (4.5)	25.2 (4.8)
Parity ≥ 1 (%)	45.2	31.7	45.7
Education (%)			
Primary school	10.6	12.2	21.0
Secondary school	41.6	44.9	60.5
Higher education	47.8	42.9	18.6
Ethnicity (%)			
Dutch, other-European	57.6	62.9	57.1
Surinamese	8.4	12.4	10.4
Turkish	7.7	7.5	16.2
Moroccan	8.1	1.2	1.9
Cape Verdian	3.7	4.6	4.8
Antillean	3.4	2.9	3.8
Others	11.0	8.5	5.8
1st Child of the same mother in study (%)	95.8	95.2	96.6
Enrolment in study in early pregnancy (%)	76.4	85.8	78.2
Ultrasound for fetal growth (%)			
Mid-pregnancy	93.8	96.6	94.0
Late pregnancy	93.9	97.0	93.7
Birth outcomes			
Birth weight (grams)	3454 (552)	3441 (559)	3251 (545)
Gestational age (weeks)*	40.0 (37.0 – 42.0)	40.0 (36.9 – 42.0)	39.9 (36.1 – 42.0)

Values are means (standard deviation) or percentages. * Median (90% range). Data were missing on height (n = 11), weight (n = 27), body mass index (n = 38), parity (n = 409), educational level (n = 218) and ethnicity (n =60).

Table 2. Associations between maternal smoking habits during pregnancy and fetal growth

Smoking category	Difference in head circumference growth (mm/week)	
	Model A	Model B
Non-smoking	*Reference*	*Reference*
Until pregnancy was known	0.05 (-0.16, 0.26)	0.10 (-0.11, 0.31)
Continued after pregnancy was known	-0.62 (-0.77, -0.46)**	-0.56 (-0.73, -0.40)**
Smoking category	Difference in abdominal circumference growth (mm/week)	
	Model A	Model B
Non-smoking	*Reference*	*Reference*
Until pregnancy was known	0.23 (-0.07, 0.52)	0.35 (0.05, 0.65)*
Continued after pregnancy was known	-0.68 (-0.90, -0.46)**	-0.58 (-0.81, -0.34)**
Smoking category	Difference in femur length growth (mm/week)	
	Model A	Model B
Non-smoking	*Reference*	*Reference*
Until pregnancy was known	0 (-0.06, 0.06)	0 (-0.05, 0.06)
Continued after pregnancy was known	-0.18 (-0.22, -0.14)**	-0.19 (-0.23, -0.14)**

Values are regression coefficients (95% confidence interval) and reflect the difference in growth (mm/week) for each characteristic.
Model A: unadjusted.
Model B: adjusted for maternal age, body mass index, height, educational level, ethnicity and parity and fetal gender.
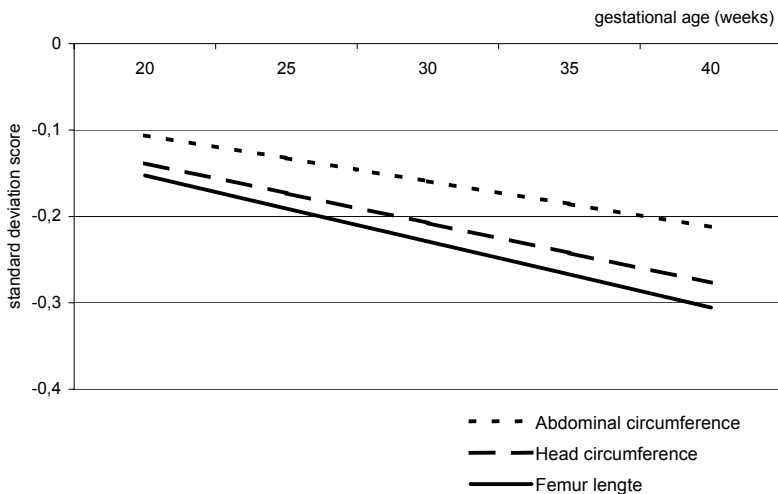*p-value < 0.05, **p-value < 0.01

Figure 1. Standard deviation scores of fetal head circumference, abdominal circumference and femur length in mothers who continued to smoke after pregnancy was known
Values are estimates based on regression models (repeated measurements) and reflect the standard deviation score for each growth characteristic in offspring of mothers who continued smoking after pregnancy was known compared to offspring of mothers who did not smoke in pregnancy.

Mid-pregnancy and late pregnancy smoking categories

Maternal smoking in mid-pregnancy was not associated with head circumference and abdominal circumference (Table 3). Smoking of less than five cigarettes per day was associated with a smaller femur length (standard deviation score -0.12 (95% CI: -0.23, -0.01)). We did not find an association between smoking five to nine cigarettes per day and femur length. A strong inverse association with femur length was found for smoking more than nine cigarettes per day (standard deviation score -0.37 (95% CI: -0.55, -0.18)).

The associations of maternal smoking in late pregnancy with fetal growth characteristics are given in Table 4. In the unadjusted models, all categories of maternal smoking were inversely associated with head circumference, abdominal circumference and femur length. For all three growth characteristics, the largest effect estimates were found for the highest smoking category, which includes mothers smoking more than 9 cigarettes per day (standard deviation scores -0.26 (95% CI: -0.45, -0.08) for head circumference, -0.25 (95% CI: -0.43, -0.06) for abdominal circumference and -0.40 (95% CI: -0.57, -0.22) for femur length).

Table 3. Associations of maternal smoking with fetal growth characteristics in mid-pregnancy (18-25 weeks)

Smoking in mid-pregnancy	Difference in head circumference (standard deviation score)		
	Model A	Model B	Model C
Non-smoking	Reference	Reference	Reference
< 5 per day	-0.14 (-0.25, -0.03)*	-0.11 (-0.22, 0.01)	-0.09 (-0.21, 0.02)
5 - 9 per day	-0.10 (-0.24, 0.04)	-0.05 (-0.20, 0.09)	-0.04 (-0.19, 0.10)
> 9 per day	-0.11 (-0.29, 0.07)	-0.04 (-0.22, 0.15)	-0.03 (-0.23, 0.16)
Smoking in mid-pregnancy	Difference in abdominal circumference (standard deviation score)		
	Model A	Model B	Model C
Non-smoking	Reference	Reference	Reference
< 5 per day	-0.10 (-0.21, 0)	-0.08 (-0.19, 0.03)	-0.06 (-0.17, 0.05)
5 - 9 per day	-0.03 (-0.16, 0.11)	0.02 (-0.12, 0.16)	-0.01 (-0.15, 0.13)
> 9 per day	0 (-0.17, 0.17)	0.06 (-0.12, 0.24)	0.07 (-0.11, 0.25)
Smoking in mid-pregnancy	Difference in femur length (standard deviation score)		
	Model A	Model B	Model C
Non-smoking	Reference	Reference	Reference
< 5 per day	-0.12 (-0.23, -0.02)*	-0.13 (-0.24, -0.02)*	-0.12 (-0.23, -0.01)*
5 - 9 per day	0 (-0.13, 0.14)	-0.01 (-0.15, 0.13)	-0.02 (-0.16, 0.12)
> 9 per day	-0.31 (-0.49, -0.14)**	-0.35 (-0.52, -0.17)**	-0.37 (-0.55, -0.18)**

Values are regression coefficients (95% confidence interval) and reflect the difference in growth characteristic (gestational age adjusted standard deviation score).
Total number of subjects: n = 4,655; non-smoking: n = 3,940; less than five per day: n = 376; five to nine per day: n = 212; more than nine per day: n = 127.
Model A: unadjusted.
Model B: adjusted for maternal body mass index and educational level.
Model B: adjusted for maternal body mass index, educational level, age, height, ethnicity and parity and fetal gender.
*p-value < 0.05, **p-value < 0.01

Table 4. Associations of maternal smoking with fetal growth characteristics in late pregnancy (≥ 25 weeks)

Smoking in late pregnancy	Difference in head circumference (standard deviation score)		
	Model A	Model B	Model C
Non-smoking	Reference	Reference	Reference
< 5 per day	-0.24 (-0.35, -0.12)**	-0.17 (-0.29, -0.05)**	-0.17 (-0.29, -0.05)**
5 - 9 per day	-0.15 (-0.29, -0.02)*	-0.08 (-0.22, 0.06)	-0.08 (-0.23, 0.06)
> 9 per day	-0.29 (-0.47, -0.11)**	-0.23 (-0.41, -0.05)*	-0.26 (-0.45, -0.08)**
Smoking in late pregnancy	Difference in abdominal circumference (standard deviation score)		
	Model A	Model B	Model C
Non-smoking	Reference	Reference	Reference
< 5 per day	-0.21 (-0.32, -0.10)**	-0.18 (-0.30, -0.06)**	-0.15 (-0.27, -0.03)*
5 - 9 per day	-0.22 (-0.35, -0.08)**	-0.17 (-0.31, -0.04)*	-0.19 (-0.32, -0.05)**
> 9 per day	-0.22 (-0.40, -0.05)*	-0.19 (-0.37, -0.01)*	-0.25 (-0.43, -0.06)**
Smoking in late pregnancy	Difference in femur length (standard deviation score)		
	Model A	Model B	Model C
Non-smoking	Reference	Reference	Reference
< 5 per day	-0.16 (-0.26, -0.05)**	-0.17 (-0.28, -0.06)**	-0.17 (-0.29, -0.06)**
5 - 9 per day	-0.28 (-0.41, -0.15)**	-0.30 (-0.43, -0.17)**	-0.29 (-0.42, -0.16)**
> 9 per day	-0.41 (-0.58, -0.24)**	-0.41 (-0.59, -0.24)**	-0.40 (-0.57, -0.22)**

Values are regression coefficients (95% confidence interval) and reflect the difference in growth characteristic (gestational age adjusted standard deviation score).

Total number of subjects: n = 4,542; non-smoking: n = 3,864; less than five per day: n = 322; five to nine per day: n = 225; more than nine per day: n = 131.

Model A: unadjusted.

Model B: adjusted for maternal body mass index and educational level.

Model B: adjusted for maternal body mass index, educational level, age, height, ethnicity and parity and fetal gender.

*p-value < 0.05, **p-value < 0.01

Discussion

This population-based prospective cohort study showed associations between maternal smoking in pregnancy and impaired growth of fetal head circumference, abdominal circumference and femur length. Differences between non-smoking mothers and mothers who continued to smoke after pregnancy was known, increased with increasing gestational age, resulting in smaller femur length from mid-pregnancy and smaller head circumference and abdominal circumference from late pregnancy.

Methodological considerations

The strength of this study is the population-based cohort with a large number of subjects studied from early pregnancy. To our knowledge, this is the largest cohort study examin-

ing the associations of maternal smoking in pregnancy with fetal growth characteristics. Follow-up information at birth was available in 93% and information about a large number of potential confounders was available in this study. Information about smoking in pregnancy at enrolment was missing in 14% of all mothers. Birth weight was 41 (95% CI: 6, 76) grams lower in the offspring of these mothers. The percentage of smoking mothers may be higher among those not included than included in the present analysis. This would probably lead to loss of power and underestimation of the estimated effects of smoking on fetal growth characteristics. Categories of maternal smoking habits at baseline were similarly distributed among those lost to follow-up and those with singleton live births as outcome. Non-response and loss to follow-up would lead to selection bias if the association of maternal smoking in pregnancy with fetal growth differs between those with and without complete data. This seems unlikely but can not be excluded.

Information about maternal smoking in pregnancy was collected by questionnaires without reference to fetal growth characteristics. Although assessing smoking habits in pregnancy by questionnaires seems to be a valid method, misclassification may occur [17]. Underreporting of maternal smoking across the various smoking categories may be present and would lead to misclassification. The estimated difference in fetal growth between the offspring of non-smoking and low to moderate smoking mothers would be overestimated if this underreporting would be selectively present among heavy smoking mothers who report low to moderate smoking. To overcome these limitations, other studies used biomarkers of tobacco exposure, including cotinine, in maternal urine samples [18, 19]. However, low correlations between cotinine and self reported smoking habits have been demonstrated [20]. Possible explanations for these low correlations include inaccurate maternal reporting of smoking habits in pregnancy, use of categories of number of cigarettes smoked in questionnaires and individual differences in inhalation, absorption and metabolism. Previous studies demonstrated that using cotinine levels is not superior to self-report in studying the effect of maternal smoking in pregnancy on birth weight [21]. We assessed smoking as categorical and not as continuous variable since data collection and data cleaning are more easily conducted in such a large cohort. Inherently, this diminishes power to identify exposure effects because information within categories is lost.

Smoking in pregnancy and fetal growth patterns

The associations of continued maternal smoking after pregnancy was known with growth of fetal head circumference, abdominal circumference and femur length were independent of potential confounders. No adverse effects on fetal growth were found in mothers who quitted smoking after their pregnancy was known. Unexpectedly, a higher fetal abdominal circumference growth was found in mothers who quitted smoking after pregnancy was known, compared to non-smokers. These findings are in line with previ-

ous studies demonstrating associations of continued maternal smoking in pregnancy with low birth weight in the offspring whereas quitting to smoke in early pregnancy is associated with a normal or even slightly increased birth weight [2, 22]. This second association seems not to be explained by an increase in body mass index [22]. The number of studies examining the effects of maternal smoking in pregnancy on fetal growth is limited [3-8]. Results from these studies are not conclusive and cannot easily be compared to our results because of the differences in study populations and growth measurements. More importantly, results from these studies were not appropriately adjusted for possible confounders. After full adjustment for life style and socio-economic status related variables, including maternal body mass index and educational level, and for known determinants of birth weight, including maternal age, height, ethnicity and parity and fetal gender, our study demonstrated strong associations of maternal smoking with femur length from mid-pregnancy onwards and of maternal smoking with head circumference and abdominal circumference from late pregnancy. These findings are in line with studies demonstrating that maternal smoking in the third trimester has the largest effect on birth weight in the offspring [23]. From the analyses with standard deviation scores as dependent variables, both the timing of the effect and the effect estimate suggest that maternal smoking in pregnancy primarily affects femur length. This supports the previously described brain-sparing effects in mild fetal growth retardation. The relatively stronger effect on femur length may indicate that maternal smoking in pregnancy or the fetal growth patterns associated with maternal smoking affect peripheral tissues and less so central tissues. Abdominal circumference was not more affected than head circumference. Further studies examining the effect of maternal smoking in pregnancy on fetal organ growth in especially late pregnancy are needed.

Study implications

Our findings suggest that maternal smoking in pregnancy leads to impaired growth of fetal head circumference, abdominal circumference and femur length. Maternal smoking until pregnancy was known and quitting thereafter did not adversely affect fetal growth. The larger effects on femur length suggest that maternal smoking in pregnancy affects primarily peripheral tissues.

References

1. Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. Bull World Health Organ 1987;65:663-737.
2. Abel EL. Smoking during pregnancy: a review of effects on growth and development of offspring. Hum Biol 1980;52:593-625.

3. Jeanty P, Cousaert E, de Maertelaer V, Cantraine F. Sonographic detection of smoking-related decreased fetal growth. J Ultrasound Med 1987;6:13-8.
4. Newnham JP, Patterson L, James I, Reid SE. Effects of maternal cigarette smoking on ultrasonic measurements of fetal growth and on Doppler flow velocity waveforms. Early Hum Dev 1990;24:23-36.
5. Vik T, Jacobsen G, Vatten L, Bakketeig LS. Pre- and post-natal growth in children of women who smoked in pregnancy. Early Hum Dev 1996;45:245-55.
6. Zaren B, Lindmark G, Bakketeig L. Maternal smoking affects fetal growth more in the male fetus. Paediatr Perinat Epidemiol 2000;14:118-26.
7. Bernstein IM, Plociennik K, Stahle S, Badger GJ, Secker-Walker R. Impact of maternal cigarette smoking on fetal growth and body composition. Am J Obstet Gynecol 2000;183:883-6.
8. Lampl M, Kuzawa CW, Jeanty P. Prenatal smoke exposure alters growth in limb proportions and head shape in the midgestation human fetus. Am J Hum Biol 2003;15:533-46.
9. Hofman A, Jaddoe VW, Mackenbach JP, Moll HA, Snijders RF, Steegers EA, et al. Growth, development and health from early fetal life until young adulthood: the Generation R Study. Paediatr Perinat Epidemiol 2004;18:61-72.
10. Jaddoe VW, Mackenbach JP, Moll HA, Steegers EA, Tiemeier H, Verhulst FC et al. The Generation R Study: study design and cohort profile. Eur J Epidemiol 2006. In press.
11. Tunon K, Eik-Nes SH, Grottum P. A comparison between ultrasound and a reliable last menstrual period as predictors of the day of delivery in 15,000 examinations. Ultrasound Obstet Gynecol 1996;8:178-85.
12. Robinson HP, Sweet EM, Adam AH. The accuracy of radiological estimates of gestational age using early fetal crown-rump length measurements by ultrasound as a basis for comparison. Br J Obstet Gynaecol 1979;86:525-8.
13. Altman DG, Chitty LS. New charts for ultrasound dating of pregnancy. Ultrasound Obstet Gynecol 1997;10:174-91.
14. Routine ultrasound screening in pregnancy: protocol. RCOG Press London, UK; 2000.
15. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. Int J Epidemiol 1999;28:964-74.
16. SAS/STAT User's Guide. Cary NSII eds.; 1998.
17. Klebanoff MA, Levine RJ, Morris CD, Hauth JC, Sibai BM, Ben Curet L, et al. Accuracy of self-reported cigarette smoking among pregnant women in the 1990s. Paediatr Perinat Epidemiol 2001;15:140-3.
18. Hebel JR, Fox NL, Sexton M. Dose-response of birth weight to various measures of maternal smoking during pregnancy. J Clin Epidemiol 1988;41:483-9.
19. Wang X, Tager IB, Van Vunakis H, Speizer FE, Hanrahan JP. Maternal smoking during pregnancy, urine cotinine concentrations, and birth outcomes. A prospective cohort study. Int J Epidemiol 1997;26:978-88.
20. English PB, Eskenazi B, Christianson RE. Black-white differences in serum cotinine levels among pregnant women and subsequent effects on infant birthweight. Am J Public Health 1994;84:1439-43.
21. Haddow JE, Knight GJ, Palomaki GE, Kloza EM, Wald NJ. Cigarette consumption and serum cotinine in relation to birthweight. BJOG 1987;94:678-81.
22. MacArthur C, Knox EG. Smoking in pregnancy: effects of stopping at different stages. Br J Obstet Gynaecol 1988;95:551-5.
23. Lieberman E, Gremy I, Lang JM, Cohen AP. Low birthweight at term and the timing of fetal exposure to maternal smoking. Am J Public Health 1994;84:1127-31.

Maternal alcohol consumption in pregnancy and the risk of low birth weight and preterm birth

4.3

Abstract

Background: The effect of low to moderate alcohol consumption in pregnancy on fetal growth is not clear. The aims of this study were to examine the associations of maternal alcohol consumption in different periods of pregnancy with the risk of low birth weight and preterm birth.

Methods: This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until adulthood. Maternal alcohol consumption was assessed by questionnaires in early pregnancy, mid-pregnancy and late pregnancy. The present analysis was based on 7,141 subjects.

Results: Compared to no alcohol consumption, continued alcohol consumption after pregnancy was known, was not associated with low birth weight (adjusted odds ratio 0.99 (95% confidence interval: 0.79, 1.24)) or preterm birth (adjusted odds ratio 0.90 (95% confidence interval: 0.69, 1.16)). For both alcohol consumption until pregnancy was known and alcohol consumption in late pregnancy (gestational age ≥ 25 weeks), using less than one alcoholic drink per day was not associated with an increased risk of low birth weight and preterm birth. Alcohol consumption of one to three drinks per day until pregnancy was known, was associated with low birth weight (adjusted odds ratio 2.28 (95% confidence interval: 0.70, 7.36)) and preterm birth (adjusted odds ratio 2.46 (95% confidence interval: 0.82, 7.38)). Due to small numbers in these categories, these effect estimates were not statistically significant.

Conclusion: Maternal consumption of less than one alcoholic drink per day in early and late pregnancy is not associated with an increased risk of low birth weight and pre-term birth. The effects of higher alcohol consumption need to be further studied.

Introduction

Excessive alcohol consumption in pregnancy is associated with various pregnancy complications including low birth weight, preterm birth, congenital anomalies, fetal alcohol syndrome and perinatal death [1-3]. Recently, it was suggested that excessive alcohol consumption in pregnancy has adverse effects on postnatal neurodevelopment in children [4]. The effect of excessive alcohol consumption in pregnancy on prenatal and postnatal growth and development can not easily be extrapolated to lower levels of alcohol consumption. Previous studies examining the effect of low or moderate alcohol consumption in pregnancy (less than one alcoholic drink per day) on birth outcomes showed inconsistent results. Several studies found adverse effects, whereas others did not find any effect or even reported beneficial effects on weight and gestational age at birth [5-8]. These inconsistent results may be due to differences in study design and in the timing and methods of assessment of alcohol consumption. Several studies used dichotomized birth outcomes including low birth weight and preterm birth [7, 9]. However, the effect on birth weight may be present on the whole birth weight range and not only on low birth weight, mostly defined as a birth weight lower than 2500 grams.

In a population-based cohort study among pregnant women and their children, we examined the associations of maternal alcohol consumption in different periods of pregnancy with continuously measured birth weight and with the risk of low birth weight and preterm birth.

Methods

Design
This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood. This study is designed to identify early environmental and genetic determinants of growth, development and health in fetal life, childhood and adulthood and has been described previously in detail [10, 11]. Briefly, the cohort includes 9,778 mothers and their children of different ethnicities living in Rotterdam, the Netherlands. Enrolment was aimed in early pregnancy (gestational age < 18 weeks) but was possible until birth of the child. Mothers were informed about the study by routine health care workers in pregnancy (midwives, obstetricians) and were enrolled in the study at their routine fetal ultrasound examination in pregnancy. Mothers who were missed in pregnancy, were approached and enrolled in the first month after birth of their child at the routine child health centers. Assessments in pregnancy, including physical examinations, fetal ultrasound examinations and questionnaires, were planned in early pregnancy (gestational age < 18 weeks), mid-pregnancy (gestational age 18-25 weeks)

and late pregnancy (gestational age ≥ 25 weeks). Mothers enrolled in early pregnancy (69%) had three assessments planned (in early, mid- and late pregnancy) whereas those enrolled in mid-pregnancy (19%) had two assessments (in mid- and late pregnancy) and those enrolled in late pregnancy (3%) had one assessment (in late pregnancy) planned. The individual time scheme of these assessments depended on the specific gestational age at enrolment [11]. Mothers enrolled after birth of their offspring (9%) had no prenatal assessments. All children were born between April 2002 and January 2006 and form a prenatally enrolled birth-cohort that is currently followed until young adulthood. Of all eligible children in the study area, 61% participated at birth in the study [11]. The study has been approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all participants.

Maternal alcohol consumption

Information about maternal alcohol consumption was obtained by postal questionnaires in early, mid- and late pregnancy [10]. In the first questionnaire, the mothers were asked whether they used any alcoholic drinks in the first three months of pregnancy (no, until pregnancy was known, continued after pregnancy was known). This questionnaire was sent to all mothers, also when they were enrolled after early pregnancy. In the second and third questionnaire, sent to the mothers in mid- and late pregnancy respectively, the mothers were asked whether they used alcoholic drinks in the past two months (no, yes). Mothers who reported in the first questionnaire to have used alcoholic drinks until pregnancy was known (n = 2,061) but still reported to use alcoholic drinks in the second or third questionnaire (n = 1,106) were reclassified into the 'continued after pregnancy was known' category. The same strategy was used for mothers who reported not to use alcoholic drinks in the first questionnaire (n = 4,072), but used alcoholic drinks in the second or third questionnaire (n = 549). Among the mothers who reported to use any alcoholic drinks, the average number of drinks was assessed in the following six categories: less than one per week; one to three per week; four to six per week; one per day; two to three per day; and more than three per day. For each questionnaire, this information was combined and reclassified into the following categories of maternal alcohol consumption: 1) never; 2) less than one drink per week; 3) one to six drinks per week; and 4) one to three drinks per day. Because of the small number of subjects using more than three alcoholic drinks per day (n = 12 in early pregnancy, n = 8 in mid-pregnancy and n = 6 in late pregnancy), this category was not included in the present analyses.

Covariates

Information about educational level, ethnicity and parity was obtained by the first questionnaire at enrolment in the study. Maternal smoking habits were assessed in each questionnaire. Maternal anthropometrics, including height and weight, were measured

without shoes and heavy clothing and body mass index was calculated (weight/height^2 (kg/m^2)) in early, mid- and late pregnancy during visits at the research center.

Birth outcomes

Date of birth, birth weight and gender were obtained from midwife and hospital registries. Gestational age was established by fetal ultrasound examination because using last menstrual period has several limitations including the large number of women who do not know their exact last menstrual period date or have irregular cycles [12].

Population for analysis

Of the total of 9,778 mothers, 91% (n = 8,880) was enrolled in pregnancy [11]. Those without information about alcohol consumption in pregnancy in the first questionnaire were excluded from the analyses (14%, n = 1,204). Of the remaining 7,676 mothers, those with twin pregnancies (n = 81), fetal deaths (n = 100) or missing birth outcomes (n = 354) were excluded. The associations of maternal alcohol consumption in pregnancy with birth outcomes were analyzed in the remaining 7,141 mothers. Of these mothers, 4% (n = 292) were second or third pregnancies in the study. Since there were no differences in results after exclusion of these subjects, they were included in the analyses presented. Analyses focused on alcohol consumption in different periods of pregnancy were restricted to mothers enrolled in early pregnancy to minimize misclassification of alcohol consumption in pregnancy period. Alcohol consumption categories within individuals were correlated between early and mid-pregnancy (Spearman correlation coefficient $r = 0.6$, p < 0.01) and between mid- and late pregnancy ($r = 0.7$, p < 0.01). Since we did not have enough mothers to assess the separate effects of each pregnancy period, analyses were focused on early pregnancy (alcohol consumption until pregnancy was known) and late pregnancy. Among mothers enrolled in early pregnancy (n = 5,533), the associations of maternal alcohol consumption until pregnancy was known with birth outcomes were assessed in those in whom information about the number of alcoholic drinks was available and did not use alcoholic drinks after pregnancy was known (n = 3,538). Among mothers enrolled in early pregnancy, information about the number of alcoholic drinks in late pregnancy was available in 83% (n = 4,589).

Data analysis

The associations of alcohol consumption habits at any time during pregnancy with continuously measured birth weight were assessed using multiple linear regression models and with low birth weight and preterm birth using multiple logistic regression models. Low birth weight was defined as birth weight below the 10th percentile in the study cohort (2850 grams) among infants born at a gestational age of 37 weeks or more. Preterm born children were excluded from the models with low birth weight as dependent variable.

Preterm birth was defined as a gestational age of less than 37 weeks at delivery. These linear and logistic regressions were adjusted for life style and socio-economic status related variables at enrolment (maternal body mass index, smoking, educational level) and additionally for known determinants of low birth weight and preterm birth (maternal ethnicity, parity and age and infant gender). In these models, smoking and body mass index were measured in the same period of pregnancy as maternal alcohol consumption. All models with low birth weight and continuously measured birth weight as dependent variable were additionally adjusted for gestational age. The same models were used to assess the associations of alcohol consumption categories in separate periods (until pregnancy was known, late pregnancy) with continuously measured birth weight, low birth weight and preterm birth. All measures of association are presented with their 95% confidence intervals (CI). All statistical analyses were performed using the Statistical Package of Social Sciences version 11.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Characteristics of the mothers are presented in Table 1. Of all mothers, 51% (n = 3,618) used alcoholic drinks in early pregnancy and 37% (n = 2,663) continued to use alcoholic drinks after pregnancy was known. In the total group, the age of mothers ranged from 15.3 to 43.3 years with a mean age of 29.8 years. The percentage of mothers with a higher educational level was highest among those who continued to use alcoholic drinks after pregnancy was known. In the total cohort, the largest ethnic groups were the Dutch and other-European (57.8%), Surinamese (9.1%), Turkish (9.1%) and Moroccan (6.5%) mothers. Among the mothers who continued to use alcoholic drinks after pregnancy was known, the percentage of Dutch mothers was higher than among the mothers who did not use alcoholic drinks in pregnancy. Mean birth weight in the singleton live births was 3417 (range 670 - 5310) grams. Among the children born with a gestational age of more than 37 weeks (n = 6,727), 10.2% was defined as low birth weight. Gestational age at birth ranged from 25.4 until 43.1 weeks with a median of 40.0 weeks. Of all singleton live births, 5.8% was born before a gestational age of 37 weeks.

Table 2 demonstrates that compared to never using alcohol in pregnancy, alcohol consumption until pregnancy was known, was not associated with a difference in continuously measured birth weight (difference -22 (95% CI: -54, 11) grams)), low birth weight (adjusted odds ratio (aOR) 1.20 (95% CI: 0.92, 1.57)) and preterm birth (aOR 0.87 (95% CI: 0.63, 1.21)). Continued alcohol consumption after pregnancy was known, was associated with continuously measured birth weight in the model adjusted for gestational age, maternal body mass index, smoking and educational level (difference 47 (95% CI: 22, 72) grams). This association disappeared after further adjustment for mater-

Table 1. Maternal characteristics according to their alcohol consumption habits in pregnancy

	Alcohol consumption in pregnancy (n = 7,141)		
	Never in pregnancy	Until pregnancy was known	Continued after pregnancy was known
	n = 3,523	n = 955	n = 2,663
Age (years)	28.4 (5.3)	29.5 (5.2)	31.6 (4.7)
Height (cm)	165.5 (7.3)	168.1 (7.1)	169.6 (6.9)
Weight (kg)	70.0 (14.4)	68.6 (12.9)	69.0 (11.8)
Body mass index (kg/m^2)	25.5 (4.9)	24.3 (4.3)	24.0 (3.8)
Parity ≥ 1 (%)	47.8	31.4	44.1
Smoking in pregnancy (%)			
No	79.4	65.8	71.3
Until pregnancy was known	4.3	17.0	10.4
Continued	16.2	17.2	18.2
Education (%)			
Primary school	19.4	6.7	5.8
Secondary school	55.0	47.6	31.5
Higher education	25.5	45.7	62.8
Ethnicity (%)			
Dutch, other-European	41.1	68.4	76.0
Surinamese	10.5	11.2	6.5
Turkish	16.7	1.8	1.7
Moroccan	12.8	0.3	0.6
Cape Verdian	4.3	5.4	3.2
Antillean	3.9	3.8	2.7
Others	10.8	9.0	9.3
1st Child of the same mother in study (%)	95.6	95.0	96.5
Enrolment in study in early pregnancy (%)	72.2	85.4	81.7
Birth outcomes			
Birth weight (grams)	3395 (552)	3351 (573)	3470 (556)
Gestational age (weeks)*	40.0 (36.7 – 42.0)	39.9 (36.4 – 41.9)	40.1 (37.0 – 42.0)

Values are means (standard deviation) or percentages. * Median (90% range). Data were missing on height (n = 11), weight (n = 27), body mass index (n = 38), parity (n = 419), smoking in pregnancy (n = 62), educational level (n = 223) and ethnicity (n =61).

nal ethnicity, parity, age and height and infant gender (difference 2 (95% CI: -23, 27) grams). Continued maternal alcohol consumption after pregnancy was known, was not associated with low birth weight (aOR 0.99 (95% CI: 0.79, 1.24)) or preterm birth (aOR 0.90 (95% CI: 0.69, 1.16)).

Among mothers who used alcoholic drinks until pregnancy was known, no differences in continuously measured birth weight were found for all categories of number of alcoholic drinks compared to mothers who never used alcoholic drinks in pregnancy

Table 2. Associations of alcohol consumption habits in pregnancy with birth outcomes

Alcohol consumption n = 7,141	Difference in birth weight (grams)[a]		
	Model A	**Model B**	**Model C**
Never in pregnancy n = 3,523	*Reference*	*Reference*	*Reference*
Until pregnancy was known n = 955	-16 (-49, 16)	-11 (-44, 22)	-22 (-54, 11)
Continued after pregnancy was known n = 2,663	46 (24, 69)**	47 (22, 72)**	2 (-23, 27)

Alcohol consumption n = 6,727 [c]	Odds ratio for low birth weight[b]		
	Model A	**Model B**	**Model C**
Never in pregnancy n = 3,308	*Reference*	*Reference*	*Reference*
Until pregnancy was known n = 896	1.18 (0.94, 1.50)	1.16 (0.91, 1.48)	1.20 (0.92, 1.57)
Continued after pregnancy was known n = 2,523	0.86 (0.71, 1.03)	0.85 (0.69, 1.04)	0.99 (0.79, 1.24)

Alcohol consumption n = 7,141	Odds ratio for preterm birth[b]		
	Model A	**Model B**	**Model C**
Never in pregnancy n = 3,523	*Reference*	*Reference*	*Reference*
Until pregnancy was known n = 955	1.01 (0.75, 1.36)	0.95 (0.69, 1.30)	0.87 (0.63, 1.21)
Continued after pregnancy was known n = 2,663	0.85 (0.69, 1.06)	0.82 (0.64, 1.05)	0.90 (0.69, 1.16)

[a] Values are regression coefficients (95% confidence interval) and number of subjects per alcohol consumption category. [b] Values are odds ratios (95% confidence interval) and number of subjects per alcohol consumption category. [c] Preterm born children excluded. Model A: unadjusted; Model B: adjusted for maternal body mass index, smoking, educational level; Model C: adjusted for maternal body mass index, smoking, educational level, height, ethnicity, parity and age and infant gender. All models with birth weight and low birth weight as dependent variable are additionally adjusted for gestational age.
**p-value < 0.01

(Table 3). No consistent associations of using less than one alcoholic drink per day with low birth weight and preterm birth were found. Associations were found between alcohol consumption of one to three drinks per day and low birth weight (aOR 2.28 (95% CI: 0.70, 7.36)) and preterm birth (aOR 2.46 (95% CI: 0.82, 7.38)). Due to the small number of subjects in these categories (n = 27 for low birth weight, n = 31 for preterm birth), these effects were not statistically significant.

Table 4 shows that alcohol consumption of less than one drink per week and one to six drinks per week in late pregnancy was not associated with a difference in continuously measured birth weight. Although statistically not significant, alcohol consumption

Table 3. Associations of alcohol consumption categories until pregnancy was known with birth outcomes

Alcohol consumption until pregnancy was known n = 3,358	Difference in birth weight (grams)[a]		
	Model A	Model B	Model C
< 1 drink per week n = 481	-23 (-67, 21)	-23 (-67, 22)	-23 (-66, 21)
1 - 6 drinks per week n = 305	15 (-39, 68)	27 (-27, 82)	5 (-49, 59)
1 - 3 drinks per day n = 31	-50 (-210, 109)	-79 (-241, 83)	-126 (-279, 27)
Alcohol consumption until pregnancy was known n = 3,138[c]	Odds ratio for low birth weight[b]		
	Model A	Model B	Model C
< 1 drink per week n = 454	0.97 (0.70, 1.35)	1.00 (0.71, 1.41)	1.00 (0.69, 1.45)
1 - 6 drinks per week n = 287	0.96 (0.64, 1.44)	0.94 (0.61, 1.46)	0.99 (0.62, 1.60)
1 - 3 drinks per day n = 27	1.65 (0.53, 5.08)	1.88 (0.60, 5.92)	2.28 (0.70, 7.36)
Alcohol consumption until pregnancy was known n = 3,358	Odds ratio for preterm birth[b]		
	Model A	Model B	Model C
< 1 drink per week n = 481	0.92 (0.60, 1.39)	0.92 (0.61, 1.43)	0.81 (0.51, 1.28)
1 - 6 drinks per week n = 305	0.97 (0.58, 1.60)	0.82 (0.48, 1.42)	0.82 (0.47, 1.43)
1 - 3 drinks per day n = 31	2.28 (0.79, 6.60)	2.31 (0.79, 6.80)	2.46 (0.82, 7.38)

[a] Values are regression coefficients (95% confidence interval) and number of subjects per alcohol consumption category. [b] Values are odds ratios (95% confidence interval) and number of subjects per alcohol consumption category. [c] Preterm born children excluded. Reference groups are mothers who do not use alcoholic drinks. Model A: unadjusted; Model B: adjusted for maternal body mass index, smoking, educational level; Model C: adjusted for maternal body mass index, smoking, educational level, height, ethnicity, parity and age and infant gender. All models with birth weight and low birth weight as dependent variable are additionally adjusted for gestational age.

of one to three drinks per day was associated with a decrease in birth weight (-118 (95% CI: -300, 65) grams). No associations were found for alcohol consumption of less than one drink per week with low birth weight and preterm birth. Using one to six alcoholic drinks per week was inversely associated with preterm birth but the estimate was not statistically significant after adjustment (aOR 0.57 (95% CI: 0.30, 1.08)). No association was found for low birth weight. Using one to three alcoholic drinks per day was associated with an increased risk of low birth weight but the effect was not significant. No association was present with preterm birth.

Table 4. Associations of alcohol consumption categories in late pregnancy with birth outcomes

Alcohol consumption in late pregnancy n = 4,589	Difference in birth weight (grams) [a]		
	Model A	Model B	Model C
< 1 drink per week n = 1,229	53 (23, 83)**	42 (11, 73)**	-6 (-36, 25)
1 - 6 drinks per week n = 436	74 (28, 119)**	54 (7, 100)*	5 (-40, 49)
1 - 3 drinks per day n = 21	-72 (-266, 122)	-87 (-276, 103)	-118 (-300, 65)

Alcohol consumption in late pregnancy n = 4,351[c]	Odds ratio for low birth weight [b]		
	Model A	Model B	Model C
< 1 drink per week n = 1,165	0.80 (0.62, 1.03)	0.85 (0.65, 1.10)	1.07 (0.80, 1.43)
1 - 6 drinks per week n = 425	0.67 (0.44, 1.03)	0.76 (0.49, 1.17)	0.90 (0.56, 1.45)
1 - 3 drinks per day n = 20	1.69 (0.46, 6.15)	1.80 (0.48, 6.78)	1.89 (0.38, 9.31)

Alcohol consumption in late pregnancy n = 4,589	Odds ratio for preterm birth [b]		
	Model A	Model B	Model C
< 1 drink per week n = 1,229	0.93 (0.69, 1.25)	0.99 (0.72, 1.36)	1.09 (0.78, 1.52)
1 - 6 drinks per week n = 436	0.44 (0.24, 0.81)**	0.49 (0.26, 0.92)*	0.57 (0.30, 1.08)
1 - 3 drinks per day n = 21	0.85 (0.11, 6.34)	0.93 (0.12, 7.03)	1.20 (0.16, 9.17)

[a] Values are regression coefficients (95% confidence interval) and number of subjects per alcohol consumption category. [b] Values are odds ratios (95% confidence interval) and number of subjects per alcohol consumption category. [c] Preterm born children excluded. Reference groups are mothers who do not use alcoholic drinks. Model A: unadjusted; Model B: adjusted for maternal body mass index, smoking, educational level; Model C: adjusted for maternal body mass index, smoking, educational level, height, ethnicity, parity and age and infant gender. All models with birth weight and low birth weight as dependent variable are additionally adjusted for gestational age. *p-value < 0.05, **p-value < 0.01

Discussion

This population-based prospective cohort study showed that maternal alcohol consumption of less than one drink per day is not associated with an increased risk of low birth weight and preterm birth. Adverse associations were found for alcohol consumption of one to three drinks per day.

Methodological considerations

The strength of this study is the population-based cohort with a large number of subjects studied from early pregnancy and the availability of a large number of potential confounders. A potential limitation of this study is that information about maternal alcohol consumption was missing in 14% of all mothers that were enrolled in pregnancy in the cohort. Birth weight was slightly lower in the offspring of mothers without information about alcohol consumption in pregnancy (difference -35 (95% CI: -71, 1) grams). Of all mothers enrolled in pregnancy, information at birth was available for 93%. Categories of maternal alcohol consumption at baseline were similarly distributed among those with singleton live birth as pregnancy outcome and those lost to follow-up. Selection bias due to non-response or loss to follow-up would be present if the associations of maternal alcohol consumption in pregnancy with the birth outcomes differ between those with and without complete data. This seems unlikely but cannot be excluded. However, relatively more incomplete data among the mothers of infants with low birth weight may have introduced less power.

Information about maternal alcohol consumption in pregnancy was collected by postal questionnaires. Using self-reported alcohol consumption may have introduced misclassification mainly because of underreporting of alcohol consumption in pregnancy [13]. If this underreporting were present across all categories of alcohol consumption, the effect estimates would be underestimated. However, if selectively mothers with heavy alcohol consumption underreport the number of drinks, the differences between no alcohol consumption and the lower categories of alcohol consumption may be overestimated. This misclassification may be prevented by using objective measures of alcohol consumption. However, current available biomarkers of alcohol consumption, including carbohydrate-deficient transferrin and gamma-glutamyl transferase, are not appropriate for assessment of light to moderate alcohol consumption [14].

Low birth weight and preterm birth

We found no adverse effects of maternal alcohol consumption of less than one drink per day in pregnancy on weight and gestational age at birth. For alcohol consumption until pregnancy was known, using one to three drinks per day was associated with an increased risk of low birth weight and preterm birth. However, due to small numbers in this category in our study, no conclusions can be drawn.

Our results are in line with previous studies that demonstrated no adverse effects of light to moderate alcohol consumption in pregnancy on the risk of low birth weight and preterm birth in the offspring [3, 7-9, 15-17]. Although these studies measured maternal alcohol consumption in different units including the number of drinks, milliliters and grams, tendencies of the relationships in these studies were similar [7-9, 15]. Lundsberg et al. demonstrated a beneficial effect of low to moderate alcohol consumption on fetal

growth retardation [7]. They suggested a J-shaped relation between maternal alcohol consumption and fetal growth retardation. Other studies that demonstrated beneficial effects of low to moderate alcohol consumption on birth weight, were not able to adjust appropriately for potential confounders [15]. Previous inconsistent results from studies assessing the associations of low to moderate maternal alcohol consumption in pregnancy with birth weight, may be explained by methodological issues including differences in assessments of maternal alcohol consumption habits, the inability to examine the effect of alcohol consumption in different periods of pregnancy and the inability to adjust for potential confounders. In our study, it turned out that continued alcohol consumption after pregnancy was known, was associated with determinants of higher birth weight (maternal height, higher educational level and Dutch ethnicity). Previous reported beneficial effects may be partly explained by residual confounding due to unmeasured socio-economic factors and life style habits related to birth weight [18]. However, like maternal smoking habits, body mass index and ethnicity, moderate alcohol consumption may also be part of the biological pathways explaining the association between maternal socio-economic status and birth weight [19]. Studies identifying the biological pathways and their etiologic fractions underlying the association between maternal socio-economic status and birth weight and whether moderate alcohol consumption in pregnancy is part of this pathway, are necessary.

Main outcomes in our study were continuously measured birth weight, low birth weight and preterm birth. These outcomes are rather crude measures of fetal growth and development. Moreover, alcohol consumption may affect fetal organ development without any effect on birth weight. Therefore further studies examining the associations of low to moderate alcohol consumption with fetal and postnatal growth and development are needed.

Study implications

Our findings suggest that maternal consumption of less than one alcoholic drink per day in early and late pregnancy is not associated with an increased risk of low birth weight and preterm birth. Before developing new public health strategies focused on alcohol consumption in pregnancy, follow-up studies are needed to assess the associations of low to moderate alcohol consumption with postnatal growth and development.

References

1. Little RE. Moderate alcohol use during pregnancy and decreased infant birth weight. Am J Public Health 1977;67:1154-6.
2. Little RE, Wendt JK. The effects of maternal drinking in the reproductive period: an epidemiologic review. J Subst Abuse 1991;3:187-204.

3. Ouellette EM, Rosett HL, Rosman NP, Weiner L. Adverse effects on offspring of maternal alcohol abuse during pregnancy. N Engl J Med 1977;297:528-30.
4. Streissguth AP, Barr HM, Sampson PD, Bookstein FL. Prenatal alcohol and offspring development: the first fourteen years. Drug Alcohol Depend 1994;36:89-99.
5. Lazzaroni F, Bonassi S, Magnani M, Calvi A, Repetto E, Serra F, et al. Moderate maternal drinking and outcome of pregnancy. Eur J Epidemiol 1993;9:599-606.
6. Parazzini F, Chatenoud L, Surace M, Tozzi L, Salerio B, Bettoni G, et al. Moderate alcohol drinking and risk of preterm birth. Eur J Clin Nutr 2003;57:1345-9.
7. Lundsberg LS, Bracken MB, Saftlas AF. Low-to-moderate gestational alcohol use and intrauterine growth retardation, low birthweight, and preterm delivery. Ann Epidemiol 1997;7:498-508.
8. Whitehead N, Lipscomb L. Patterns of alcohol use before and during pregnancy and the risk of small-for-gestational-age birth. Am J Epidemiol 2003;158:654-62.
9. Mariscal M, Palma S, Llorca J, Perez-Iglesias R, Pardo-Crespo R, Delgado-Rodriguez M. Pattern of alcohol consumption during pregnancy and risk for low birth weight. Ann Epidemiol 2005. doi:10.1016/j.annepidem. 2005.07.058
10. Hofman A, Jaddoe VW, Mackenbach JP, Moll HA, Snijders RF, Steegers EA, et al. Growth, development and health from early fetal life until young adulthood: the Generation R Study. Paediatr Perinat Epidemiol 2004;18:61-72.
11. Jaddoe VW, Mackenbach JP, Moll HA, Steegers EA, Tiemeier H, Verhulst FC et al. The Generation R Study: study design and cohort profile. Eur J Epidemiol 2006. In press.
12. Tunon K, Eik-Nes SH, Grottum P. A comparison between ultrasound and a reliable last menstrual period as predictors of the day of delivery in 15,000 examinations. Ultrasound Obstet Gynecol 1996;8:178-85.
13. da Costa Pereira A, Olsen J, Ogston S. Variability of self reported measures of alcohol consumption: implications for the association between drinking in pregnancy and birth weight. J Epidemiol Community Health 1993;47:326-30.
14. van Pelt J, Leusink GL, van Nierop PW, Keyzer JJ. Test characteristics of carbohydrate-deficient transferrin and gamma-glutamyltransferase in alcohol-using perimenopausal women. Alcohol Clin Exp Res 2000;24:176-9.
15. McDonald AD, Armstrong BG, Sloan M. Cigarette, alcohol, and coffee consumption and prematurity. Am J Public Health 1992;82:87-90.
16. Virji SK. The relationship between alcohol consumption during pregnancy and infant birthweight. An epidemiologic study. Acta Obstet Gynecol Scand 1991;70:303-8.
17. Albertsen K, Andersen AM, Olsen J, Gronbaek M. Alcohol consumption during pregnancy and the risk of preterm delivery. Am J Epidemiol 2004;159:155-61.
18. Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. Bull World Health Organ 1987;65:663-737.
19. Kramer MS. Socioeconomic determinants of intrauterine growth retardation. Eur J Clin Nutr 998;52: S29-32;

Cardiovascular risk factors in the offspring

5

Fetal growth characteristics and
left cardiac structure in early infancy

5.1

Abstract

Background: It has been suggested that left ventricular hypertrophy partly originates in early life. The aim of this study was to examine the associations of fetal growth characteristics and characteristics at birth with left cardiac structure in early infancy.

Methods: This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until adulthood. Fetal growth (head circumference, abdominal circumference and femur length) was assessed by ultrasound examinations in mid-pregnancy (gestational age 18 - 25 weeks) and late pregnancy (gestational age ≥ 25 weeks). Echocardiographic measurements were performed in a subgroup of 791 infants aged 7 (95% range: 4.4 - 12.3) weeks.

Results: We did not find associations of mid-pregnancy growth characteristics with postnatal left cardiac structures. In late pregnancy, fetal abdominal circumference was positively associated with left ventricular mass (0.33 (95% confidence interval: 0.06, 0.61) grams per standard deviation score) and aortic root diameter (0.13 (95% confidence interval: 0.03, 0.22) mm per standard deviation score). Birth weight was positively associated with aortic root diameter (0.21 (95% confidence interval: 0.07, 0.34) mm per standard deviation score). These effect estimates were adjusted for age, gender and infant weight and length.

Conclusion: Our findings suggest that smaller fetal size in late pregnancy is associated with smaller left ventricular mass and aortic root diameter in early infancy. Further studies should examine whether these changes persist with increasing age.

Introduction

The fetal origins hypothesis postulates that an adverse fetal environment leads to developmental adaptations that permanently program the fetus' structure, physiology and metabolism [1]. This programming would be in favour of short-term survival and would lead to fetal growth retardation and low birth weight. Long-term effects of this programming would be detrimental and lead to cardiovascular disease. This hypothesis is supported by epidemiological studies that have consistently demonstrated associations of low birth weight with coronary heart disease and its risk factors [2, 3]. The most commonly studied risk factors are blood pressure and total cholesterol levels [4, 5].

Left ventricular hypertrophy is a strong and independent risk factor of cardiovascular morbidity and mortality [6]. An adverse environment in fetal or early postnatal life may affect left ventricular growth and development since the human heart has its highest growth rate in these periods [7]. Only few studies examined the associations of weight in early life with left ventricular mass in adulthood [8, 9]. These studies demonstrated that low weight in infancy is associated with an increased left ventricular mass in adulthood and suggested that left ventricular hypertrophy originates at least partly in early life. To our knowledge, no previous studies have examined the associations of fetal growth characteristics with postnatal left ventricular mass in healthy children or adults. This information may be relevant for identifying critical periods in fetal life for left ventricular growth and development.

Therefore, we examined in a population-based prospective cohort study the associations of fetal growth characteristics and characteristics at birth with left cardiac structure in early infancy.

Methods

Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood. This study is designed to identify early environmental and genetic determinants of growth, development and health in fetal life, childhood and adulthood and has been described previously in detail [10, 11]. Briefly, the cohort includes 9,778 mothers and their children of different ethnicities living in Rotterdam, the Netherlands. Enrolment was aimed in early pregnancy (gestational age < 18 weeks) but was possible until birth of the child. Mothers were informed about the study by routine health care workers in pregnancy (midwives, obstetricians) and were enrolled at their routine fetal ultrasound examination in pregnancy. Mothers who were missed in pregnancy, were approached and enrolled in the first month after birth

of their child at the routine child health centers. Assessments in pregnancy, including physical examinations, fetal ultrasound examinations and questionnaires, were planned in early pregnancy (gestational age < 18 weeks), mid-pregnancy (gestational age 18 - 25 weeks) and late pregnancy (gestational age ≥ 25 weeks). Mothers enrolled in early pregnancy (69%) had three assessments planned (in early, mid- and late pregnancy) whereas those enrolled in mid-pregnancy (19%) had two assessments (in mid- and late pregnancy) and those enrolled in late pregnancy (3%) had one assessment (in late pregnancy) planned [11]. Mothers enrolled after birth of their offspring (9%) had no prenatal assessments. All children were born between April 2002 and January 2006 and form a prenatally enrolled birth-cohort that is currently followed until young adulthood. Additional detailed assessments are conducted in a subgroup study, comprising 1,232 Dutch pregnant women and their children, referred to as the Generation R Focus Study. The subgroup is ethnic homogeneous to exclude confounding or effect modification by ethnicity. Of all approached women, 80% was enrolled in this subgroup study in late pregnancy. Postnatal echocardiograms were planned in infants aged 6 weeks in this sub-group. The study was approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all participants.

Fetal growth and birth characteristics

Fetal ultrasound examinations were carried out at the visits in one of the research centers in early, mid- and late pregnancy. These fetal ultrasound examinations were used for both establishing gestational age and assessing fetal growth characteristics [12]. Pregnancy dating curves were constructed on subjects in the study with complete data on gestational age measured by ultrasound and last menstrual period. Crown-rump length was used for pregnancy dating until a gestational age of 12 weeks (crown-rump length smaller than 80 mm) and biparietal diameter was used for pregnancy dating thereafter (gestational age from 12 weeks onwards, biparietal diameter larger than 20 mm) [13, 14]. Fetal growth measurements used for the present study included head circumference, abdominal circumference and femur length in mid- and late pregnancy, measured to the nearest mm using standardized ultrasound procedures [15]. Early pregnancy was not included since these fetal ultrasound examinations were primarily performed to establish gestational age. Gestational age adjusted standard deviation scores were constructed for all fetal growth measurements. These were based on reference growth curves from the whole study population. The median (95% range) gestational age for the fetal ultrasound examinations in early, mid- and late pregnancy was 13.1 (9.3 - 17.5) weeks, 20.5 (18.4 - 23.3) weeks and 30.4 (27.9 - 33.0) weeks, respectively. Date of birth, birth weight and gender were obtained from midwife and hospital registries.

Left cardiac structure

Two-dimensional M-mode and Doppler echocardiograms were performed using Kretz Voluson 530D equipment in the infants. The examination was carried out in a quiet room with the baby quietly awake in supine position. One echocardiographer performed the vast majority (86%) of these measurements. Two other echocardiographers performed the other measurements. In a parasternal long-axis view, left ventricular end diastolic diameter (LVEDD), interventricular septal thickness (IVST), left ventricular posterior wall thickness (LVPWT), aortic root diameter (AO) and left atrial diameter (LAD) were measured using methods recommended by the American Society of Echocardiography [16]. Left ventricular mass was computed by use of the formula derived by Devereux et al.: Left ventricular mass = 0.80 * 1.04 ((IVST + LVEDD +LVPWT)^3 – (LVEDD)^3)+ 0.6 [17].

Covariates

Postnatal anthropometrics were measured without clothes at the same visit as the echocardiograms. Weight (grams) was measured using an electronic scale and length (cm) was measured in supine position using a neonatometer.

Population for analysis

In total 1,232 mothers were enrolled in the Generation R Focus Study in the prenatal period. The present analysis was limited to singleton live births (n = 1,208). Of these singleton live births, 77% (n = 927) participated in the postnatal assessments. Echocardiograms were performed in 86% (n = 801) of these infants. Missing echocardiograms in the others infants were mainly due to crying or unavailability of the equipment or echocardiographer. Infants who had a postnatal echocardiogram did not differ from the singleton live births who did not have a postnatal echocardiogram (n = 407) in mean birth weight (difference 18 (95% confidence interval: –48, 83) grams). Infants with congenital heart disease were excluded (n = 10) from the present analysis, leaving 791 subjects.

Data analysis

Associations of fetal growth characteristics with left cardiac structures (left ventricular mass, left atrium diameter, aortic root diameter) were assessed using multiple linear regression models. These fetal growth characteristics included standard deviation scores for head circumference, abdominal circumference and femur length measured in mid- and late pregnancy and birth weight. These models were first adjusted for gender and age (Model A) and additionally for current weight and current length (Model B). All models with birth weight as independent variable were also adjusted for gestational age. The simultaneous effects of fetal and postnatal growth were examined by observed mean values of left ventricular mass and aortic root diameter in tables with tertiles of abdominal circumference in late pregnancy, birth weight and current weight. Multiple

linear regression models adjusted for gender and age were considered to be tests for trends within these tertile strata. All measures of association are presented with their 95% confidence intervals (CI). All statistical analyses were performed using the Statistical Package of Social Sciences version 11.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Characteristics of infants and their mothers are presented in Table 1. The percentage of boys was 52%. Fetal head circumference and birth weight were larger in boys than in girls. Femur length was larger in girls. The median age in infants at their visit was 7.0 (95% range: 4.4 – 12.3) weeks. Weight, length and left cardiac dimensions were lower in girls than in boys.

Table 1. Subject characteristics

	Boys (n = 413)	Girls (n = 378)
Pregnancy and birth characteristics		
Maternal age (years)	31.7 (3.9)	31.8 (3.8)
Mid-pregnancy growth (standard deviation score)		
Head circumference	0.24 (1.22)	-0.20 (1.23)
Abdominal circumference	0.16 (0.97)	0.11 (0.96)
Femur length	-0.16 (1.17)	0.02 (1.22)
Late pregnancy growth (standard deviation score)		
Head circumference	0.37 (1.23)	-0.05 (1.13)
Abdominal circumference	0.18 (1.03)	0.16 (0.98)
Femur length	-0.25 (1.19)	0.02 (1.22)
Birth weight (grams)	3528 (541)	3476 (517)
Gestational age (weeks)	40.3 (35.6 – 42.6)	40.3 (36.1 – 42.4)
Postnatal characteristics		
Age at visit (weeks)	7.0 (4.4 – 12.8)	6.9 (4.4 – 11.6)
Weight (grams)	5094 (754)	4747 (631)
Length (cm)	58 (3)	56 (3)
Left cardiac structure		
Left ventricular mass (grams)	16.1 (3.1)	14.6 (2.9)
Left atrium diameter (mm)	16.9 (1.9)	16.6 (1.9)
Aortic root diameter (mm)	12.0 (1.2)	11.5 (1.0)

Values are means (standard deviation) or medians (95% range).
Of the total group, data were missing on mid-pregnancy head circumference (n = 11), mid-pregnancy abdominal circumference (n = 15), mid-pregnancy femur length (n = 15), late pregnancy head circumference (n = 15), late pregnancy abdominal circumference (n = 18), late pregnancy femur length (n = 14), weight (n =3), length (n = 12), left ventricular mass (n = 102), left atrium diameter (n = 31) and aortic root diameter (n = 39).

Table 2 presents the regression coefficients of mid-pregnancy growth characteristics with left cardiac structures. All effect estimates were small and not statistically significant. No consistent tendencies were found. Table 3 presents the associations of late pregnancy growth characteristics and birth weight with left cardiac structures. All fetal growth characteristics were positively associated with left ventricular mass after adjustment for age and gender. After additional adjustment for current weight and length, only abdominal circumference was positively associated with left ventricular mass (0.33 (95% CI: 0.06, 0.61) grams per standard deviation score). Although not statistically significant in the fully adjusted model, birth weight was positively associated with left ventricular mass (0.28 (95% CI: -0.12, 0.69) grams per standard deviation score). Of all growth characteristics in late pregnancy, only femur length was associated with left atrium diameter (-0.16 (95% CI: -0.30, -0.02) mm per standard deviation score). In the full adjusted model, the association between birth weight and left atrium diameter was inverse but not significant (-0.11 (95% CI: -0.35, 0.14) mm per standard deviation score). All growth characteristics in late pregnancy were positively associated with aortic root diameter. After adjustment for length and current weight, only statistically significant associations were found for abdominal circumference (0.13 (95% CI: 0.03, 0.22) mm per standard deviation score) and birth weight (0.21 (95% CI: 0.07, 0.34) mm per standard deviation score).

Table 2. Fetal growth characteristics in mid-pregnancy and left cardiac structures

	Left ventricular mass (grams)	
Mid-pregnancy growth characteristic	**Model A**	**Model B**
Head circumference	0.09 (-0.13, 0.31)	0.08 (-0.12, 0.29)
Abdominal circumference	0.15 (-0.15, 0.44)	0.03 (-0.24, 0.31)
Femur length	0.04 (-0.19, 0.26)	-0.03 (-0.24, 0.19)
	Left atrium diameter (mm)	
Mid-pregnancy growth characteristic	**Model A**	**Model B**
Head circumference	-0.01 (-0.02, 0.01)	-0.01 (-0.02, 0.01)
Abdominal circumference	0.01 (0, 0.03)	0.01 (-0.01, 0.03)
Femur length	0.01 (0, 0.02)	0.01 (-0.01, 0.02)
	Aortic root diameter (mm)	
Mid-pregnancy growth characteristic	**Model A**	**Model B**
Head circumference	0 (-0.01, 0.01)	0 (-0.01, 0.01)
Abdominal circumference	0.01 (0, 0.02)	0 (-0.01, 0.01)
Femur length	0 (-0.01, 0.01)	0 (-0.01, 0.01)

Values are regression coefficients (95% confidence interval) and reflect the difference in left cardiac structure per standard deviation score increase in growth characteristic. Model A: adjusted for age and gender; Model B: adjusted for age, gender, current weight and current length.

Table 3. Fetal growth characteristics in late pregnancy and at birth and left cardiac structures

Late pregnancy growth characteristic	Left ventricular mass (grams)	
	Model A	Model B
Head circumference	0.41 (0.17, 0.64)**	0.12 (-0.07, 0.39)
Abdominal circumference	0.65 (0.38, 0.92)**	0.33 (0.06, 0.61)*
Femur length	0.26 (0.03, 0.49)*	0 (-0.23, 0.23)
Birth weight	1.00 (0.73, 1.27)**	0.28 (-0.12, 0.69)
Late pregnancy growth characteristic	Left atrium diameter (mm)	
	Model A	Model B
Head circumference	-0.01 (-0.15, 0.12)	-0.07 (-0.21, 0.07)
Abdominal circumference	0.04 (-0.12, 0.20)	-0.05 (-0.22, 0.11)
Femur length	-0.08 (-0.21, 0.05)	-0.16 (-0.30, -0.02)*
Birth weight	0.17 (0, 0.33)*	-0.11 (-0.35, 0.14)
Late pregnancy growth characteristic	Aortic root diameter (mm)	
	Model A	Model B
Head circumference	0.12 (0.04, 0.20)**	0.06 (-0.02, 0.14)
Abdominal circumference	0.20 (0.11, 0.29)**	0.13 (0.03, 0.22)**
Femur length	0.09 (0.01, 0.17)*	0.03 (-0.05, 0.11)
Birth weight	0.32 (0.23, 0.41)**	0.21 (0.07, 0.34)**

Values are regression coefficients (95% confidence interval) and reflect the difference in left cardiac structure per standard deviation score increase in growth characteristic. Model A: adjusted for age and gender; Model B: adjusted for age, gender, current weight and current length. Models with birth weight as independent variable are additionally adjusted for gestational age.
*p-value < 0.05, **p-value < 0.01

Table 4 presents the simultaneous effects of late pregnancy abdominal circumference, birth weight and current weight on left ventricular mass. Current weight was positively associated with left ventricular mass in all tertiles of late pregnancy abdominal circumference and birth weight. Within the lowest and middle tertiles of current weight, positive associations of late pregnancy abdominal circumference with left ventricular mass were found (both tests for trend: p < 0.05). Birth weight was only associated with left ventricular mass in the highest tertile of current weight (test for trend: p < 0.01). The simultaneous effects of late pregnancy abdominal circumference, birth weight and current weight on aortic root diameter are given in Table 5. Positive associations were found between late pregnancy abdominal circumference and aortic root diameter among subjects in the lowest and middle tertiles of current weight (tests for trend p < 0.01 for lowest tertile, p < 0.05 for middle tertile). In all tertiles of current weight, birth weight was associated with aortic root diameter.

Table 4. Left ventricular mass (grams) in tertiles of late pregnancy abdominal circumference and birth weight, stratified by current weight

	Current weight tertile				
	Lowest	Middle	Highest	Total	
Late pregnancy abdominal circumference tertile					
Lowest	13.4 (106)	15.0 (64)	16.5 (56)	14.6 (226)	$P_{trend} < 0.01$
Middle	14.1 (71)	15.7 (82)	16.8 (64)	15.5 (217)	$P_{trend} < 0.01$
Highest	14.4 (51)	15.8 (70)	16.8 (106)	16.0 (227)	$P_{trend} < 0.01$
Total	13.9 (228)	15.6 (216)	16.7 (226)	15.4 (670)	
	$P_{trend} < 0.05$	$P_{trend} < 0.05$	$P_{trend} = 0.2$		
	Current weight tertile				
	Lowest	Middle	Highest	Total	
Birth weight tertile					
Lowest	13.7 (143)	15.6 (56)	16.2 (30)	14.5 (229)	$P_{trend} < 0.01$
Middle	13.9 (76)	15.4 (90)	16.3 (60)	15.5 (226)	$P_{trend} < 0.01$
Highest	15.4 (15)	15.8 (75)	17.1 (138)	16.5 (228)	$P_{trend} < 0.05$
Total	13.9 (234)	15.6 (221)	16.8 (228)	15.4 (683)	
	$P_{trend} = 0.11$	$P_{trend} = 0.24$	$P_{trend} < 0.01$		

Values are observed means of left ventricular mass (number of subjects). Test for trends are based on multiple linear regression models within tertiles adjusted for gender and age. Models with birth weight as independent variable are additionally adjusted for gestational age.

Table 5. Aortic root diameter (mm) in tertiles of late pregnancy abdominal circumference and birth weight, stratified by current weight

	Current weight tertile				
	Lowest	Middle	Highest	Total	
Late pregnancy abdominal circumference tertile					
Lowest	11.1 (116)	11.6 (67)	12.2 (60)	11.5 (243)	$P_{trend} < 0.01$
Middle	11.4 (80)	11.8 (90)	12.2 (69)	11.8 (239)	$P_{trend} < 0.01$
Highest	11.3 (51)	11.9 (76)	12.1 (123)	11.8 (250)	$P_{trend} < 0.01$
Total	11.3 (247)	11.7 (233)	12.2 (252)	11.7 (732)	
	$P_{trend} < 0.01$	$P_{trend} = 0.05$	$P_{trend} = 0.6$		
	Current weight tertile				
	Lowest	Middle	Highest	Total	
Birth weight tertile					
Lowest	11.2 (154)	11.6 (60)	12.0 (36)	11.4 (250)	$P_{trend} < 0.01$
Middle	11.4 (83)	11.7 (99)	12.2 (67)	11.7 (249)	$P_{trend} < 0.01$
Highest	11.3 (16)	11.9 (76)	12.2 (152)	12.1 (247)	$P_{trend} < 0.01$
Total	11.3 (253)	11.7 (238)	12.2 (255)	11.7 (746)	
	$P_{trend} < 0.01$	$P_{trend} < 0.05$	$P_{trend} < 0.01$		

Values are observed means of aortic root diameter (number of subjects). Test for trends are based on multiple linear regression models within tertiles adjusted for gender and age. Models with birth weight as independent variable are additionally adjusted for gestational age.

Discussion

Our population-based prospective cohort study showed positive associations of late pregnancy abdominal circumference and birth weight with left ventricular mass and aortic root diameter in early infancy. These associations were independent of current weight and length.

Methodological considerations

To our knowledge, this is the largest prospective cohort study examining the associations of fetal growth characteristics with left cardiac structures. Postnatal follow-up echocardiograms were successfully obtained in 65% of all singleton live births of mothers enrolled in pregnancy in the Generation R Focus Study. Birth weight was similar between infants with and without a postnatal echocardiogram The effect estimates would be biased if the associations of fetal growth characteristics with left cardiac structures differ between those included and not included in the present analyses. This seems unlikely. The missing values of left cardiac structure among infants who did participate in the other postnatal (anthropometric) assessments were mainly due to crying or unavailability of equipment or echocardiographer. These missing values are not expected to lead to biased results.

Fetal growth and cardiac structure

Our findings indicate that subjects who are smaller in late pregnancy or at birth have smaller left ventricular mass and aortic root diameter in early infancy. These associations were still present after adjustment for weight and length in infancy. However, adjustment for weight and length in infancy did affect almost all effect estimates towards smaller or even inverse associations. Confounding may be the case, but we cannot exclude overadjustment due to strong positive associations between growth characteristics in fetal life, at birth and in infancy. Therefore, the effect estimates were given both unadjusted and adjusted for weight and length in infancy. An inverse association was found between late pregnancy femur length and left atrium diameter. We cannot explain this finding since it was not consistent with the other results.

We did not find any associations between mid-pregnancy growth characteristics or late pregnancy head circumference with postnatal cardiac structures. Relatively small abdominal circumference in late pregnancy is a measure of fetal growth retardation in the second half of pregnancy. Therefore, an adverse fetal environment in the second half of pregnancy may affect growth and development of the left cardiac and aortic structures.

Several biological pathways may explain the associations between fetal growth retardation and cardiac changes. Fetal growth retardation is associated with fetal blood

flow redistribution to the brain at expense of the abdomen and lower limbs [18, 19]. This redistribution is associated with an increase in fetal left ventricular blood flow and increased peripheral arterial resistance [18]. These fetal changes in haemodynamic stimuli may lead to persistent structural left ventricular changes. Previous studies demonstrated that fetal growth retardation is associated with changes in left ventricular diastolic filling patterns, which may be attributed to increased ventricular stiffness [20]. Another biological pathway may include hormonal changes associated with fetal growth retardation that directly affect heart growth. Growth hormone, insulin like growth factor-1 and insulin stimulate ventricular growth and are decreased in fetal growth retardation [21]. An adverse fetal environment may also directly affect cardiac growth. Recently, it was suggested that maternal smoking in pregnancy is associated with adaptations in fetal cardiac dimensions and volumes [22]. Further studies are needed to identify these pathways.

From our study, it is not known whether and to what extend the cardiac changes we demonstrated in early infancy persist in later life. However, previous studies suggest that these cardiac changes have consequences for later life. A relatively smaller left ventricle and aortic root may lead to insufficient cardiac functioning for increasing metabolic demands in postnatal life. The heart may respond to these increased demands by growth and remodelling. Since the number of heart cells is established largely in fetal life, this remodelling would lead to adaptation and growth of existing cells. This process may be in favour of short-term cardiac functioning but may eventually lead to increased left ventricular mass or even left ventricular hypertrophy. Our hypothesis is supported by studies in children and adults [23-25]. Recently, it was demonstrated that low birth weight was, independently of current weight and height associated with persistent smaller total coronary heart diameter, aortic root diameter and left ventricular outflow tract diameter in children aged 9 years [23]. Increased growth rate and weight change in childhood, which is present in most low birth weight children, are associated with increased left ventricular mass [24, 25]. Studies in adults demonstrated that low weight in infancy was associated with increased left ventricular mass in adults [8, 9]. Follow-up studies in our children or in other studies examining the effect of growth in different periods of life on left cardiac structure, are needed to confirm these findings.

Study implications

Our findings suggest that smaller fetal size in late pregnancy is associated with smaller left ventricular mass and aortic root diameter in early infancy. Further studies are needed to examine the biological pathways leading to these cardiac changes and to examine whether these cardiac changes persist with increasing age.

References

1. Barker DJ. Fetal origins of coronary heart disease. BMJ 1995;311:171-4.
2. Frankel S, Elwood P, Sweetnam P, Yarnell J, Smith GD. Birthweight, body-mass index in middle age, and incident coronary heart disease. Lancet 1996;348:1478-80.
3. Curhan GC, Willett WC, Rimm EB, Spiegelman D, Ascherio AL, Stampfer MJ. Birth weight and adult hypertension, diabetes mellitus, and obesity in US men. Circulation 1996;94:3246-50.
4. Huxley R, Neil A, Collins R. Unravelling the fetal origins hypothesis: is there really an inverse association between birthweight and subsequent blood pressure? Lancet 2002;360:659-65.
5. Huxley R, Owen CG, Whincup PH, Cook DG, Colman S, Collins R. Birth weight and subsequent cholesterol levels: exploration of the "fetal origins" hypothesis. JAMA 2004;292:2755-64.
6. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. N Engl J Med 1990;322:1561-6.
7. Ishii A, Tatsunami S, Satoh I, Honma T, Hamada H, Yago N. Growth dynamics of the heart from perinatal period to childhood. J Perinat Med 1990;18:459-63.
8. Vijayakumar M, Fall CH, Osmond C, Barker DJ. Birth weight, weight at one year, and left ventricular mass in adult life. Br Heart J 1995;73:363-7.
9. Zureik M, Bonithon-Kopp C, Lecomte E, Siest G, Ducimetiere P. Weights at birth and in early infancy, systolic pressure, and left ventricular structure in subjects aged 8 to 24 years. Hypertension 1996;27:339-45.
10. Hofman A, Jaddoe VW, Mackenbach JP, Moll HA, Snijders RF, Steegers EA, et al. Growth, development and health from early fetal life until young adulthood: the Generation R Study. Paediatr Perinat Epidemiol 2004;18:61-72.
11. Jaddoe VW, Mackenbach JP, Moll HA, Steegers EA, Tiemeier H, Verhulst FC et al. The Generation R Study: study design and cohort profile. Eur J Epidemiol 2006. In press.
12. Tunon K, Eik-Nes SH, Grottum P. A comparison between ultrasound and a reliable last menstrual period as predictors of the day of delivery in 15,000 examinations. Ultrasound Obstet Gynecol 1996;8:178-85.
13. Robinson HP, Sweet EM, Adam AH. The accuracy of radiological estimates of gestational age using early fetal crown-rump length measurements by ultrasound as a basis for comparison. BJOG 1979;86:525-8.
14. Altman DG, Chitty LS. New charts for ultrasound dating of pregnancy. Ultrasound Obstet Gynecol 1997;10:174-91.
15. Routine ultrasound screening in pregnancy: protocol RCOG. RCOG Press London, UK; 2000.
16. Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, et al. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. J Am Soc Echocardiogr 1989;2:358-67.
17. Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, et al. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. Am J Cardiol 1986;57:450-8.
18. al-Ghazali W, Chita SK, Chapman MG, Allan LD. Evidence of redistribution of cardiac output in asymmetrical growth retardation. BJOG 1989;96:697-704.
19. Severi FM, Rizzo G, Bocchi C, D'Antona D, Verzuri MS, Arduini D. Intrauterine growth retardation and fetal cardiac function. Fetal Diagn Ther 2000;15:8-19.
20 Rizzo G, Arduini D, Romanini C, Mancuso S. Doppler echocardiographic assessment of atrioventricular velocity waveforms in normal and small-for-gestational-age fetuses. BJOG 1988;95:65-9.

21. Jensen RB, Chellakooty M, Vielwerth S, Vaag A, Larsen T, Greisen G, et al. Intrauterine growth retardation and consequences for endocrine and cardiovascular diseases in adult life: does insulin-like growth factor-I play a role? Horm Res 2003;60:136-48.
22. Lampl M, Kuzawa CW, Jeanty P. Growth patterns of the heart and kidney suggest inter-organ collaboration in facultative fetal growth. Am J Hum Biol 2005;17:178-94.
23. Jiang B, Godfrey KM, Martyn CN, Gale CR. Birth weight and cardiac structure in children. Pediatrics 2006;117:e257-61.
24. Urbina EM, Gidding SS, Bao W, Pickoff AS, Berdusis K, Berenson GS. Effect of body size, ponderosity, and blood pressure on left ventricular growth in children and young adults in the Bogalusa Heart Study. Circulation 1995;91:2400-6.
25. Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL. Children born small for gestational age: do they catch up? Pediatr Res 1995;38:267-71.

Maternal smoking in pregnancy and blood pressure development in the offspring

5.2

Abstract

Background: Maternal smoking in pregnancy seems to be associated with increased blood pressure in the offspring in childhood. The aim of this study was to examine the effect of maternal smoking in pregnancy on the longitudinal blood pressure development from childhood into adulthood.

Methods: Blood pressure was annually measured from 1975 to 1993 and in 2002 in a cohort of 350 subjects, aged 5-19 years at baseline. Pregnancy and birth data were obtained through questionnaires sent to the parents.

Results: The median follow-up period was 23.6 (95% range 3.1 - 26.9) years. Children of mothers who smoked in pregnancy showed a higher annual change of 0.27 (95% confidence interval: 0.10, 0.44) mmHg per year for systolic blood pressure and 0.17 (95% confidence interval: 0.04, 0.30) mmHg per year for diastolic blood pressure compared to the offspring of mothers who did not smoke in pregnancy. This difference in annual change led to a higher systolic and diastolic blood pressure from early adulthood onwards. Adjustment for adult risk factors including current weight, current smoking and current alcohol consumption, did not materially change the effect estimates and their confidence intervals. After adjustment for socio-economic status, the association showed the same direction but was only significant in men. Additional adjustment for birth weight did not change the effect estimates.

Conclusion: Maternal smoking in pregnancy is associated with a steeper increase in systolic and diastolic blood pressure in the offspring leading to a higher blood pressure from early adulthood onwards. The underlying causal pathway may involve other mechanisms than low birth weight.

Introduction

Recent studies suggested that maternal smoking in pregnancy is associated with increased blood pressure in the offspring [1-3]. These studies were conducted in children until the age of 9 years. Since blood pressure under the age of 10 years tends to track from childhood into adulthood, these findings suggest that maternal smoking in pregnancy may have lifelong consequences for the risk of high blood pressure and the development of cardiovascular disease [4-6]. To our knowledge, no studies have been conducted examining the effect of maternal smoking in pregnancy on longitudinal blood pressure development in the offspring from early childhood until adulthood. Furthermore, current studies are not conclusive about the causal pathway leading from maternal smoking in pregnancy to increased blood pressure in the offspring in childhood [1-3]. Maternal smoking in pregnancy is an important determinant of low birth weight in Western countries. Low birth weight is associated with an increased blood pressure in adulthood and may explain at least part of the proposed association of maternal smoking in pregnancy with increased blood pressure in the offspring [7].

The aim of the present study was to examine the association of maternal smoking in pregnancy with the longitudinal blood pressure development in the offspring from childhood until adulthood. We also examined whether the causal pathway from maternal smoking in pregnancy to increased blood pressure in the offspring includes low birth weight.

Methods

Design and study population

All subjects aged 5 years and older living in two districts in Zoetermeer, a suburban town in the Netherlands, were invited to participate in the Epidemiological Preventive Organization Zoetermeer (EPOZ) Study between 1975 and 1978 [8]. This is a population-based study on risk indicators for chronic disease. Of the 5,670 eligible children aged 5-19 years, 4,649 (response 82%) were included. From this group, a random sample of 596 children was selected for an annual follow-up study on the natural history of cardiovascular risk factors and their determinants. Complete data were available for 425 fathers and 454 mothers at baseline. The children visited the research center annually in the same month of the year, preferably at the same time of the day, between 1975 and 1993 and again in 2002. The response for these annual visits gradually declined to 81% (n = 483) in 1993 and 61% (n = 362) in 2002. The median number of visits for the present analysis is 15 (range 2 - 19) and the median follow-up time is 23.6 (95% range 3.1 - 26.9) years.

Measurements

Blood pressure was measured using a Hawksley random-zero sphygmomanometer (Lancing, Sussex) according to a standardized protocol on the left brachial artery of a sitting subject after a resting period of 15 minutes [9]. A cuff of 23 by 10 or 14 cm was used depending on the arm circumference. The largest cuff was used in children over 10 years of age. Diastolic blood pressure was taken at the 5th Korotkoff phase. The mean of two consecutive measurements was used in the analyses. Body height and weight were measured without shoes and heavy clothing and body mass index was calculated (weight/height^2 (kg/m^2). Information on life style factors including smoking habits and alcohol consumption was obtained through questionnaires at each visit. The same methods were used in both parents at baseline.

Pregnancy and birth data

A questionnaire to obtain pregnancy and birth data was sent to the parents of the children in 1993. This questionnaire included questions about birth weight (grams), birth length (cm), gestational age (weeks) and maternal smoking in pregnancy (no, yes). Of the 483 subjects in the study in 1993, the addresses of 33 parents could not be found and 2 children had died during follow-up. Of the 448 questionnaires sent, 353 were completed and returned to the investigators (response 79%). In 2002, missing pregnancy and birth data were completed. Information on maternal smoking in pregnancy was known in 350 subjects.

Data analysis

The main outcome of the present study was repeatedly measured blood pressure in the offspring. Since repeated blood pressure measurements within children are dependent observations, unbalanced repeated measurement analysis was used. First, the association between age and blood pressure was modeled using fractional polynomials resulting in a best fitting model including both age and age$^{-0.5}$ [10]. The intercept and slope were used as random effects. Second, since the effect of maternal smoking in pregnancy on blood pressure depended on age (p-value for interaction < 0.05), the interaction term maternal smoking*age was added to this model. The basic model for the influence of maternal smoking in pregnancy on systolic and diastolic blood pressure development over time in the offspring, adjusted for gender, can be written as:

blood pressure = β_0 + β_1*smoking in pregnancy + β_2* gender + β_3*age + β_4*age$^{-0.5}$ + β_5*smoking in pregnancy*age.

In this model 'β_0 + β_1*smoking in pregnancy + β_2* gender' reflects the intercept and 'β_3*age + β_4*age$^{-0.5}$ + β_5*smoking in pregnancy*age' reflects the slope of the change in

blood pressure over time. The term β_5 reflects the difference in annual blood pressure change between the offspring of mothers who smoked in pregnancy and of mothers who did not smoke in pregnancy. This basic model was subsequently adjusted for current risk factors in childhood and adulthood (height, weight, body mass index, current smoking habits and current alcohol consumption, socio-economic status) and covariates related to birth (birth weight, gestational age). In an additional analysis, birth weight, current weight and their interaction were added to the basic model as proposed by Lucas et al [11]. These models were proposed to identify the different associations of birth weight, current weight and their interaction with blood pressure. All measures of association are presented with their 95% confidence interval (CI). All statistical analyses were performed using the Statistical Analysis System (SAS), including the Proc Mixed module for unbalanced repeated measurements [12].

Results

Characteristics of the subjects at baseline and at their latest visit are presented in Table 1. Data on maternal smoking in pregnancy were available for 192 men and 158 women. The mean age at baseline was 13 years. The number of offspring of mothers who smoked in pregnancy was 71 (20.2%).
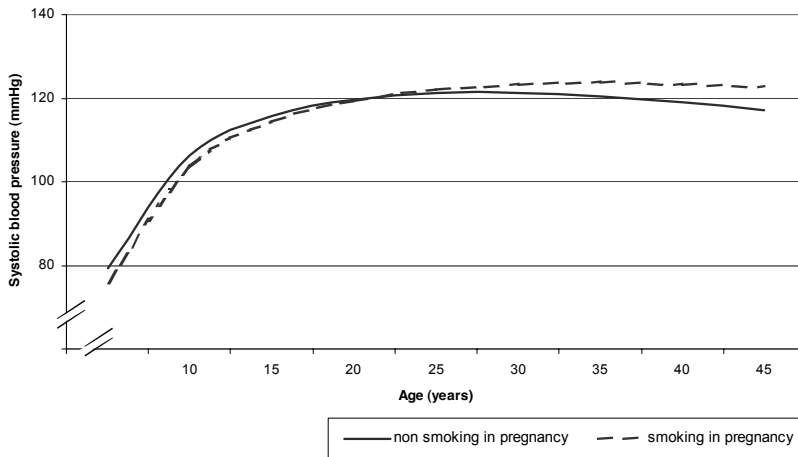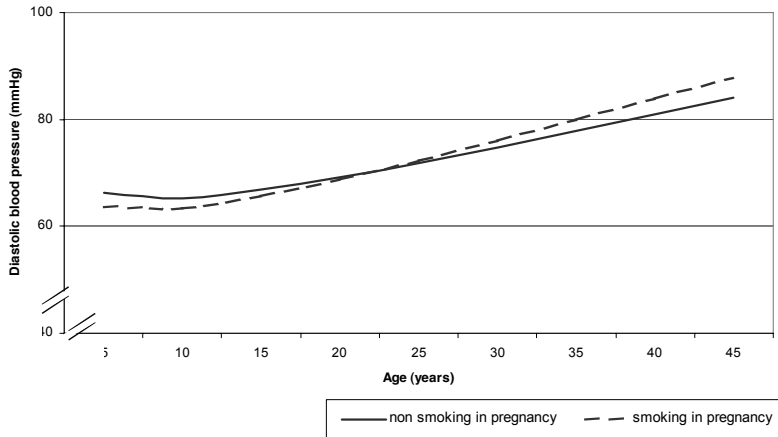

Figure 1. Maternal smoking in pregnancy and systolic blood pressure development in the offspring
Values are based on the following repeated measurements regression model:
Systolic blood pressure $= \beta_0 + \beta_1*$smoking in pregnancy $+ \beta_2*$ gender $+ \beta_3*$age $+ \beta_4*$age$^{-0,5} + \beta_5*$smoking in pregnancy*age.

Figure 2. Maternal smoking in pregnancy and diastolic blood pressure development in the offspring
Values are based on the following repeated measurements regression model:
Diastolic blood pressure = $\beta_0 + \beta_1$*smoking in pregnancy + β_2* gender + β_3*age + β_4*age$^{-0.5}$ + β_5*smoking in pregnancy*age

Figures 1 and 2 show the systolic and diastolic blood pressure development in the offspring from mothers who smoked and who did not smoke in pregnancy. Systolic and diastolic blood pressure of offspring of mothers who smoked in pregnancy were somewhat lower in early childhood but showed a higher annual blood pressure change leading to an increased absolute systolic and diastolic pressure from early adulthood. The increase in annual change was 0.27 (95% CI: 0.10, 0.44) mmHg per year for systolic blood pressure and 0.17 (95% CI: 0.04, 0.30) mmHg per year for diastolic blood pressure compared to the offspring of mothers who did not smoke in pregnancy.

Adjustment for risk factors in current and early life

Table 2 presents the effect of different potential confounders on the association of maternal smoking in pregnancy with the annual change in systolic and diastolic blood pressure. Maternal smoking in pregnancy was associated with a steeper increase in annual blood pressure change leading to an increased absolute systolic and diastolic blood pressure from early adulthood in all models. Current body mass index and height had similar effects on the estimated difference in annual change for both systolic and for diastolic blood pressure as current weight and the results are therefore not presented separately. Current smoking habits, including the number of cigarettes, and alcohol consumption did not materially change the effect estimates. Highest followed educational level was the only current factor that influenced the association. After adjustment for highest followed educational level the association of maternal smoking in pregnancy with systolic blood pressure had the same direction in men and women and was signifi-

Table 1. General characteristics of men and women at their first and last visit

	Men (n = 192)	Women (n = 158)
Baseline (1975-1978)		
Age (years)	13.3 (4.2)	12.9 (4.0)
Height (cm)	157.4 (21.9)	152.3 (17.1)
Weight (kg)	47.1 (17.8)	44.2 (14.7)
Body mass index (kg/m^2)	18.1 (2.7)	18.4 (3.1)
Systolic blood pressure (mmHg)	116.0 (16.0)	112.3 (13.9)
Diastolic blood pressure (mmHg)	68.0 (10.6)	66.7 (9.7)
Birth weight (grams)	3520 (643)	3335 (551)
Gestational age (weeks)	39.1 (1.6)	39.1 (1.5)
Maternal smoking in pregnancy (%)	19.3	22.2
Maternal systolic blood pressure (mmHg)	125.8 (16.5)	124.4 (16.7)
Maternal diastolic blood pressure (mmHg)	79.5 (11.2)	78.2 (11.1)
Last visit		
Age (years)	34.5 (6.8)	33.8 (6.9)
Height (cm)	181.8 (6.8)	168.7 (7.4)
Weight (kg)	83.0 (13.7)	71.2 (14.8)
Body mass index (kg/m^2)	25.1 (3.6)	25.0 (4.6)
Systolic blood pressure (mmHg)	125.1 (13.1)	115.6 (11.8)
Diastolic blood pressure (mmHg)	80.1 (9.4)	74.7 (9.7)
Smoking (%)	42.7	30.1
Alcohol use (%)	86.5	81.7
Highest followed education level (%)		
Elementary / secondary school	20.9	26.4
Lower / intermediate vocational training	54.0	51.8
Higher vocational training / university	25.1	21.8
Follow-up (years)*	23.7 (6.0 – 26.5)	23.4 (3.1 - 26.9)

Values are expressed as mean (standard deviation) in case of continuous variables and as percentage in case of categorical variables.
* Median (95% range)

cant for men (0.33 (95% CI: 0.09, 0.57) mmHg per year) but not for women (0.11 (95% CI: -0.13, 0.35) mmHg per year). After adjustment for highest educational level the estimated differences for annual change in diastolic blood pressure were 0.16 (95% CI: -0.02, 0.34) mmHg per year in men and 0.14 (95% CI: -0.07, 0.35) mmHg per year in women. These differences between men and women are not shown in Table 2. Adjustment for factors in early life did not change the estimated difference in slope to a large extent.

Table 2. Difference in annual blood pressure change in the offspring of mothers who smoked in pregnancy compared to those of mothers who did not smoke in pregnancy

Model adjustment	Systolic blood pressure (mmHg / year)	Diastolic blood pressure (mmHg / year)
Maternal smoking, age, gender	0.27 (0.10, 0.44)**	0.17 (0.04, 0.30)*
Adjustment for current risk factors		
+ current weight	0.21 (0.06, 0.36)**	0.14 (0.03, 0.26)*
+ current smoking (no, yes)	0.26 (0.09, 0.43)**	0.16 (0.03, 0.29)*
+ current number of cigarettes	0.20 (0, 0.39)*	0.17 (0.04, 0.34)*
+ current alcohol consumption (no, yes)	0.26 (0.09, 0.43)**	0.16 (0.03, 0.29)*
+ educational level	0.16 (-0.03, 0.36)	0.11 (-0.03, 0.25)
Adjustment for early life factors		
+ birth weight	0.28 (0.10, 0.45)**	0.18 (0.05, 0.31)**
+ gestational age	0.27 (0.09, 0.45)**	0.17 (0.04, 0.31)*
+ maternal blood pressure	0.25 (0.07, 0.42)**	0.17 (0.04, 0.30)*

Values are regression coefficients (95% confidence interval) based on repeated measurements analysis and reflect the difference in annual blood pressure change between the offspring of mothers who smoked in pregnancy and the offspring of mothers who did not smoke in pregnancy.
*p-value < 0.05
**p-value < 0.01

Table 3. Difference in annual blood pressure change in the offspring of mothers who smoked in pregnancy compared to those of mothers who did not smoke in pregnancy adjusted for birth weight and current weight

Model adjustment	Systolic blood pressure (mmHg / year)	Diastolic blood pressure (mmHg / year)
Maternal smoking, age, gender, birth weight	0.28 (0.10, 0.45)**	0.18 (0.05, 0.31)**
+ current weight	0.21 (0.07, 0.36)**	0.14 (0.03, 0.26)*
+ current weight and interaction between birth weight and current weight	0.20 (0.06, 0.35)**	0.15 (0.03, 0.27)*

Values are regression coefficients (95% confidence interval) based on repeated measurements analysis and reflect the difference in annual blood pressure change between the offspring of mothers who smoked in pregnancy and the offspring of mothers who did not smoke in pregnancy.
*p-value < 0.05
**p-value < 0.01

Birth weight as mediating factor

The mean birth weight was 3298 (SD 521) grams in the offspring of mothers who smoked in pregnancy and 3470 (SD 626) grams in the offspring of mothers who did not smoke in pregnancy. Table 3 presents the difference in annual change in systolic and diastolic blood pressure in the offspring of mothers who smoked in pregnancy compared to the offspring of mothers who did not smoke in pregnancy after adjustment for birth weight, current weight and their interaction according to the models proposed by Lucas et al [11]. Maternal smoking in pregnancy was significantly associated with an increased

annual change in systolic and diastolic blood pressure development in the offspring in all models. Birth weight was negatively and current weight was positively associated with systolic and diastolic blood pressure development in all models. However, the association between birth weight and systolic blood pressure was only significant in the model including current weight (increase of 2.85 (95% CI: 1.34, 4.35) mmHg per kilogram decrease in birth weight). The association between birth weight and diastolic blood pressure was inverse but non-significant in all models.

Discussion

This 27-year follow-up study showed an association between maternal smoking in pregnancy and a higher annual change in systolic and diastolic blood pressure in the offspring leading to increased blood pressure levels from early adulthood. The association was not explained by low birth weight, suggesting that other causal pathways may be involved.

Strengths and limitations

The strength of this study is the long-term prospective follow-up with a large number of measurements performed in each subject. This large number increases the accuracy of the estimation of the true underlying blood pressure of each subject. A potential limitation of this study is that it is based on 62% of the subjects of the original cohort. Blood pressure at baseline was similar in those lost to follow-up and those not lost to follow-up (113.6 (SD 14.9) and 114.6 (SD 15.1) for systolic blood pressure, respectively). This loss to follow-up would only lead to selection bias if the association between maternal smoking in pregnancy and blood pressure development differs between those lost to follow-up and those not lost to follow-up. This seems unlikely. Information about maternal smoking in pregnancy was retrospectively obtained by questionnaires sent to the mothers. Of the 353 completed and returned questionnaires 99% (n = 350) had information about maternal smoking in pregnancy. Although questionnaires seem to be a valid method for retrospective information collection on maternal smoking in pregnancy, misclassification and information bias may occur [13]. The mothers were not aware of this specific research question at the moment of completing the questionnaires. The pregnancy data were obtained without reference to the blood pressure data and a large number of the blood pressure measurements have been conducted after returning the questionnaire about maternal smoking in pregnancy. Therefore, any misclassification is most likely to be random and would tend to underestimate the effect size.

Maternal smoking in pregnancy and blood pressure in the offspring

The most important finding of this study is the association between maternal smoking in pregnancy and a higher annual change in systolic and diastolic blood pressure development in the offspring. This finding was independent of most potential confounding variables. Socio-economic status, measured as highest followed educational level, was the only confounder that changed the estimated effect size. However, even after adjusting for socio-economic status, the association showed the same trend and was still significant in men. In adults, socio-economic status is associated with hypertension and cardiovascular diseases [14]. Intermediate variables in the association between socio-economic status and cardiovascular disease seem to include body mass index, alcohol consumption and smoking habits [14, 15]. Since adjusting for these variables separately did not change the estimated effect size in our subjects, other mechanisms may be important. Residual confounding by other life-style habits associated with socio-economic status including diet may be the case. However, since socio-economic status was associated with maternal smoking in pregnancy and may be a correlate of maternal smoking, over-adjustment cannot be excluded (Table 4).

The finding of the association between maternal smoking in pregnancy with blood pressure development in the offspring is in line with other studies [1-3]. These studies showed associations between maternal smoking in pregnancy and blood pressure in children aged till 9 years. However, in contrast to these studies, in our study absolute sys-

Table 4. Distribution of educational level in the offspring of mothers who smoked in pregnancy and in those of mothers who did not smoke in pregnancy

Educational level	No maternal smoking in pregnancy	Maternal smoking in pregnancy
Elementary / secondary school (%)	20.3	35.6
Lower / intermediate vocational training (%)	52.8	53.3
Higher vocational training / university (%)	26.9	11.1

tolic and diastolic blood pressure were increased only from young adulthood onwards in the offspring of mothers who smoked in pregnancy compared to the offspring of mothers who did not smoke in pregnancy. Another difference is that in these other studies maternal smoking in pregnancy was also associated with an increased body mass index in young children. Adjustment for body mass index did not change the effect estimates in our study. Differences in other risk factors or life style habits between populations may explain the differences between these study results.

Underlying mechanisms

In adults, the biological mechanisms underlying the associations between smoking and blood pressure have not been clarified yet. Although smoking is associated with an acute rise in blood pressure, some epidemiological studies have suggested that smoking is associated with a lower blood pressure in adults [16, 17]. Furthermore, smoking cessation is associated with an increase in blood pressure in adults [18, 19]. These effects seem to be independent of current weight and body mass index. However, smoking is associated with hypertension, atherosclerosis and coronary heart disease in the longer term [20]. Suggested mechanisms leading from smoking to hypertension and atherosclerosis in the long run include platelet aggregation, impaired lipoprotein metabolism, inflammatory responses and arterial stiffness [21-24]. Although maternal smoking in pregnancy may not lead to an increased blood pressure at younger age in the offspring, the cumulative effects of these separate sub-clinical mechanisms may lead to an increased blood pressure in adolescence and eventually hypertension. Studies examining the associations between maternal smoking in pregnancy and these potential sub-clinical mechanisms have not been conducted yet.

Based on the association between low birth weight and cardiovascular disease in adulthood, it has been hypothesized that an adverse fetal environment leads to fetal adaptation mechanisms in growth and development [25]. These adaptation mechanisms would lead to fetal growth retardation and low birth weight in short term and to cardiovascular disease in later life. Maternal smoking in pregnancy may lead to an adverse fetal environment both by direct fetal exposure to nicotine and by fetal undernutrition or placental dysfunction due to associated dietary habits in women who smoke in pregnancy. According to this hypothesis, fetal growth retardation and low birth weight could be intermediates in the association between maternal smoking in pregnancy and blood pressure in the offspring. Although maternal smoking in pregnancy was associated with low birth weight in our study, the association between maternal smoking in pregnancy and blood pressure development in the offspring did not disappear in models adjusting for low birth weight. This finding suggests that the causal pathway from maternal smoking in pregnancy to increased blood pressure in the offspring does not include birth weight per se. However, since fetal growth retardation due to maternal smoking in pregnancy does not have to lead to low birth weight if the fetus was supposed to grow on the upper percentiles, misclassification cannot be excluded. A small study in humans has demonstrated that fetal exposure to nicotine is associated with smaller kidneys [26]. It has been suggested that small kidneys are associated with increased blood pressure in later life [27]. Therefore, fetal organ development may be permanently changed due to fetal exposure to nicotine despite only small changes in birth weight. Further studies examining the effect of maternal smoking in pregnancy on fetal and early postnatal

renal growth and vascular development and subsequent blood pressure could identify part of the underlying mechanisms.

Study implications

Although the additional annual increase in blood pressure change between the offspring of mothers who smoked in pregnancy and of mothers who did not smoke in pregnancy seems to be small, the life-course change will be larger. The additional annual change would lead to an additional 4 – 6 mmHg increase in systolic blood pressure between the ages of 10 and 30 years, and even larger in later years. This effect size suggests important implications for public health. Therefore other studies, especially focused on the underlying mechanisms are necessary.

References

1. Morley R, Leeson Payne C, Lister G, Lucas A. Maternal smoking and blood pressure in 7.5 to 8 year old offspring. Arch Dis Child 1995;72:120-4.
2. Blake KV, Gurrin LC, Evans SF, Beilin LJ, Landau LI, Stanley FJ, et al. Maternal cigarette smoking during pregnancy, low birth weight and subsequent blood pressure in early childhood. Early Hum Dev 2000;57:137-47.
3. Lawlor DA, Najman JM, Sterne J, Williams GM, Ebrahim S, Davey Smith G. Associations of parental, birth, and early life characteristics with systolic blood pressure at 5 years of age: findings from the Mater-University study of pregnancy and its outcomes. Circulation 2004;110:2417-23.
4. Labarthe DR, Eissa M, Varas C. Childhood precursors of high blood pressure and elevated cholesterol. Annu Rev Public Health 1991;12:519-41.
5. Nelson MJ, Ragland DR, Syme SL. Longitudinal prediction of adult blood pressure from juvenile blood pressure levels. Am J Epidemiol 1992;136:633-45.
6. Berenson GS, Srinivasan SR, Bao W, Newman WP, 3rd, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. N Engl J Med 1998;338:1650-6.
7. Uiterwaal CS, Anthony S, Launer LJ, Witteman JC, Trouwborst AM, Hofman A, et al. Birth weight, growth, and blood pressure: an annual follow-up study of children aged 5 through 21 years. Hypertension 1997;30:267-71.
8. Valkenburg HA, Hofman A, Klein F, Groustra FN. An epidemiological study of risk indicators for cardiovascular diseases (EPOZ). I. Blood pressure, serum cholesterol level, Quetelet-index and smoking habits in an open population aged 5 years and older. Ned Tijdschr Geneeskd 1980;124:183-9.
9. Hofman A, Valkenburg HA. Determinants of change in blood pressure during childhood. Am J Epidemiol 1983;117:735-43.
10. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. Int J Epidemiol 1999;28:964-74.
11. SAS/STAT User's Guide. Cary NSII eds.; 1998.
12. Tomeo CA, Rich-Edwards JW, Michels KB, Berkey CS, Hunter DJ, Frazier AL, et al. Reproducibility and validity of maternal recall of pregnancy-related events. Epidemiology 1999;10:774-7.
13. Colhoun HM, Hemingway H, Poulter NR. Socio-economic status and blood pressure: an overview analysis. J Hum Hypertens 1998;12:91-110.

14. Bell AC, Adair LS, Popkin BM. Understanding the role of mediating risk factors and proxy effects in the association between socio-economic status and untreated hypertension. Soc Sci Med 2004;59:275-83.

15. Green MS, Jucha E, Luz Y. Blood pressure in smokers and nonsmokers: epidemiologic findings. Am Heart J 1986;111:932-40.

16. Omvik P. How smoking affects blood pressure. Blood Press 1996;5:71-7.

17. Janzon E, Hedblad B, Berglund G, Engstrom G. Changes in blood pressure and body weight following smoking cessation in women. J Intern Med 2004;255:266-72.

18. Lee DH, Ha MH, Kim JR, Jacobs DR, Jr. Effects of smoking cessation on changes in blood pressure and incidence of hypertension: a 4-year follow-up study. Hypertension 2001;37:194-8.

19. Ockene IS, Miller NH. Cigarette smoking, cardiovascular disease, and stroke: a statement for health-care professionals from the American Heart Association. American Heart Association Task Force on Risk Reduction. Circulation 1997;96:3243-7.

20. Fusegawa Y, Goto S, Handa S, Kawada T, Ando Y. Platelet spontaneous aggregation in platelet-rich plasma is increased in habitual smokers. Thromb Res 1999;93:271-8.

21. Muscat JE, Harris RE, Haley NJ, Wynder EL. Cigarette smoking and plasma cholesterol. Am Heart J 1991;121:141-7.

22. Bazzano LA, He J, Muntner P, Vupputuri S, Whelton PK. Relationship between cigarette smoking and novel risk factors for cardiovascular disease in the United States. Ann Intern Med 2003;138:891-7.

23. Stefanadis C, Tsiamis E, Vlachopoulos C, Stratos C, Toutouzas K, Pitsavos C, et al. Unfavorable effect of smoking on the elastic properties of the human aorta. Circulation 1997;95:31-8.

24. Lampl M, Kuzawa CW, Jeanty P. Growth patterns of the heart and kidney suggest inter-organ collaboration in facultative fetal growth. Am J Hum Biol 2005;17:178-94.

25. Brenner BM, Chertow GM. Congenital oligonephropathy and the etiology of adult hypertension and progressive renal injury. Am J Kidney Dis 1994;23:171-5.

Maternal smoking in pregnancy and cholesterol development in the offspring

5.3

Abstract

Background: Maternal smoking in pregnancy is associated with overweight and increased blood pressure in the offspring and may thereby increase the risk for cardiovascular disease in adulthood. The aim of the present study was to examine whether maternal smoking in pregnancy is also associated with development of cholesterol levels from childhood into adulthood.

Methods: Total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, weight and height were measured annually from 1975 to 1993 and in 2002 in a cohort of 350 subjects, aged 5 - 19 years at baseline. Pregnancy and birth data were obtained through questionnaires sent to the parents.

Results: The median follow-up period was 23.6 (95% range 3.1 - 26.9) years. Children of mothers who smoked in pregnancy showed a higher annual change in total cholesterol of 0.01 mmol/l per year (95% confidence interval: 0, 0.02) compared to children whose mothers did not smoke in pregnancy. The association between maternal smoking in pregnancy and cholesterol development was restricted to children with moderate overweight. HDL-cholesterol and LDL-cholesterol showed trends towards a decrease and increase, respectively, in children of mothers who smoked in pregnancy compared to children whose mothers did not smoke in pregnancy. Adjustment for potential confounders including current smoking habits, alcohol consumption, socio-economic status and birth weight did not materially change these effect estimates.

Conclusion: This study demonstrates for the first time that maternal smoking in pregnancy is associated with an increased childhood rise in total cholesterol levels and a trend towards an adverse lipoprotein profile in the offspring. The effect of maternal smoking in pregnancy on total cholesterol development was restricted to the offspring with moderate overweight.

Introduction

The fetal origins hypothesis postulates that an adverse fetal environment, especially fetal undernutrition, leads to developmental adaptations that permanently program the fetus' structure, physiology and metabolism [1]. This programming would be in favour of short-term survival and would lead to fetal growth retardation and low birth weight. Long-term effects of this programming would be detrimental and lead to cardiovascular diseases and their risk factors. This hypothesis has gradually been modified into a more general developmental plasticity model in which various fetal and postnatal exposures would lead to programming responses [2].

Recent systematic reviews of epidemiological studies suggested only small effects of low birth weight on both blood pressure and blood cholesterol in later life [3, 4]. An explanation for these small effect sizes and the discrepancy with findings from animal studies may be that low birth weight is not an appropriate measure of an adverse fetal environment. Studies examining the effect of directly measured adverse fetal exposures instead of only birth weight on diseases in later life may reveal stronger associations. Maternal smoking in pregnancy is the most important determinant of low birth weight in Western countries and may lead to persistent adverse developmental changes due to direct fetal exposure to nicotine and the associated maternal life style and dietary habits. It has been demonstrated previously that maternal smoking in pregnancy is associated with obesity and higher blood pressure in childhood [5, 6]. These effects were independent of birth weight and may predispose the individual to the development of cardiovascular disease. To our best knowledge, it is currently unknown whether maternal smoking in pregnancy is associated with cholesterol levels in the offspring.

Therefore, we studied the associations between maternal smoking in pregnancy with development of total cholesterol, HDL-cholesterol and LDL-cholesterol levels from childhood into adulthood.

Methods

Design and study population

All subjects aged 5 years and older living in two districts in Zoetermeer, a suburban town in the Netherlands, were invited to participate in the Epidemiological Preventive Organization Zoetermeer (EPOZ) Study between 1975 and 1978 [7]. This is a population-based study on risk indicators for chronic disease. Of the 5,670 eligible children aged 5-19 years, 4,649 (response 81%) were included. From this group, a random sample of 596 children was selected for an annual follow-up study on the natural history of cardiovascular risk factors and their determinants. Complete data were available for 425

fathers and 454 mothers at baseline. The children visited the research center annually in the same month of the year, preferably at the same time of the day, between 1975 and 1993 and again in 2002. The response for these annual visits gradually declined to 81% (n = 483) in 1993 and 61% (n = 362) in 2002. The median number of visits for the present analysis is 15 (range 2 - 19) and the median follow-up time is 23.6 (95% range 3.1 - 26.9) years.

Measurements

The annual measurements were performed in the same month of the year for each individual. Blood samples were drawn by antecubital venipuncture for cholesterol measurements from the start of the study. Measurements of high-density lipoprotein (HDL)-cholesterol were started in 1979 and of low-density lipoprotein (LDL)-cholesterol were started in 1984. Height and weight were measured without shoes and heavy clothing and body mass index was calculated (weight/height^2 (kg/m^2)). Information on life style factors including smoking habits and alcohol consumption was obtained through questionnaires at each visit. The same methods were used in both parents at baseline.

Laboratory analysis

The laboratory analysis of lipoprotein-cholesterol concentrations for this study is described in detail elsewhere [8]. Briefly, serum total cholesterol was measured with an automated enzymatic method at baseline and from 1983 to 1993 with a modified reagent (CHOD/PAP High Performance, Boehringer Mannheim, FRG) [9]. The standard deviation of duplicate serum cholesterol measurements stored at –20°C for up to 4 years did not exceed 3.0% and did not show a significant drift. Measurements of HDL-cholesterol (from 1979) and LDL-cholesterol (from 1984) were performed by the same method after precipitation. A phosphotungstate method with a minor modification was used for HDL-cholesterol measurements and polyvinylsulphate (Boehringer Mannheim, FRG) was used for LDL-cholesterol [10, 11]. All measurements were carried out at the Department of Epidemiology & Biostatistics at the Erasmus Medical Center, Rotterdam, the Netherlands. This department participated in the Dutch National Cholesterol standardization program (KCA foundation) from 1977 and in the lipid standardization program of the World Health Organization (WHO) Regional Lipid Reference Center in Prague, Czechoslovakia from 1978. During the baseline period, quality control was indirectly checked on the CDC protocol by monthly comparison with cholesterol determination using the Abell-Kendall method [12]. Accuracy and precision of total cholesterol and HDL-cholesterol measurement were within acceptable limits (CDC/WHO) over the entire period. From 1989, automated analyses were carried out on a Technicon Auto Analyser-II system (Technicon Instruments, Tarrytown, NY, USA) initially and on a Kone Specific Analyzer (Kone Instruments, Espoo, Finland) using frozen (–20°C) serum samples. In 2002, serum

total cholesterol was measured by an automated enzymatic procedure using Roche CHOD-PAP reagent kit. HDL-cholesterol was measured with the Roche CHOD-PAP direct HDL-cholesterol assay using PEG-modified enzymes and dextran sulphate.

Pregnancy and birth data

A questionnaire to obtain pregnancy and birth data was sent to the parents of the children in 1993. This questionnaire included questions about birth weight (grams) and maternal smoking in pregnancy (no, yes). Of the 483 subjects in the study in 1993, the addresses of 33 parents could not be found and 2 children had died during follow-up. Of the 448 questionnaires sent, 353 were completed and returned to the investigators (response 79%). Information about maternal smoking in pregnancy was known in 350 subjects.

Data analysis

The main outcomes of the present study were repeatedly measured total cholesterol, HDL-cholesterol and LDL-cholesterol levels. Since these measurements within children are dependent observations, unbalanced repeated measured analysis was used. The associations between age and the main outcomes were modelled using fractional polynomials resulting in the best fitting model including both age and $age^{-0.5}$ [13]. The intercept and slope were used as random effects in all models. Since the effect of maternal smoking in pregnancy on total cholesterol depended on age (p-value for interaction 0.04), the interaction term maternal smoking*age was added to the model with total cholesterol as outcome. This interaction was also tested but not significant in the models with HDL-cholesterol and LDL-cholesterol as outcome. The basic model for the influence of maternal smoking in pregnancy on total cholesterol development over time in the offspring, adjusted for gender, can be written as:

Total cholesterol = β_0 + β_1*smoking in pregnancy + β_2*gender + β_3*age + β_4*$age^{-0.5}$ + β_5*smoking in pregnancy*age.

In this model 'β_0 + β_1*smoking in pregnancy + β_2*gender' reflects the level of total cholesterol and 'β_3*age + β_4*$age^{-0.5}$ + β_5*smoking in pregnancy*age' reflects the slope of the change in total cholesterol over time. The term β_5 reflects the difference in overall annual change of total cholesterol between the offspring of mothers who smoked in pregnancy and of mothers who did not smoke in pregnancy. The similar models without the interaction term 'β_5*smoking in pregnancy*age' were used for HDL-cholesterol and LDL-cholesterol as outcomes. Main interest in these models was in β_1, which reflects the difference in HDL-cholesterol and LDL-cholesterol between the offspring of mothers who smoked in pregnancy and of mothers who did not smoke in pregnancy and

which is assumed to be constant over time. All models were subsequently adjusted for potential confounders in childhood and adulthood (current height, weight, body mass index, current smoking habits, current alcohol consumption, socio-economic status and birth weight). Since there was a significant interaction between smoking in pregnancy and body mass index (p-value for interaction 0.03), the modifying effect of body mass index on the association of maternal smoking in pregnancy with the annual change in cholesterol development was examined in an additional stratified analysis within three body mass index standard deviation score groups (< -1, -1 to 1, > 1). These scores were based on national reference curves and were standardized by age and gender. Moderate overweight was defined as body mass index standard deviation score > 1. All measures of association are presented with their 95% confidence intervals (CI). All statistical analyses were performed using the Statistical Analysis System (SAS), including the Proc Mixed module for unbalanced repeated measurements [14].

Results

Table 1 demonstrates the characteristics of the subjects at baseline and at their latest visit. Data on maternal smoking in pregnancy were available for 192 men and 158 women. The number of offspring of mothers who smoked in pregnancy was 71 (20.2%).
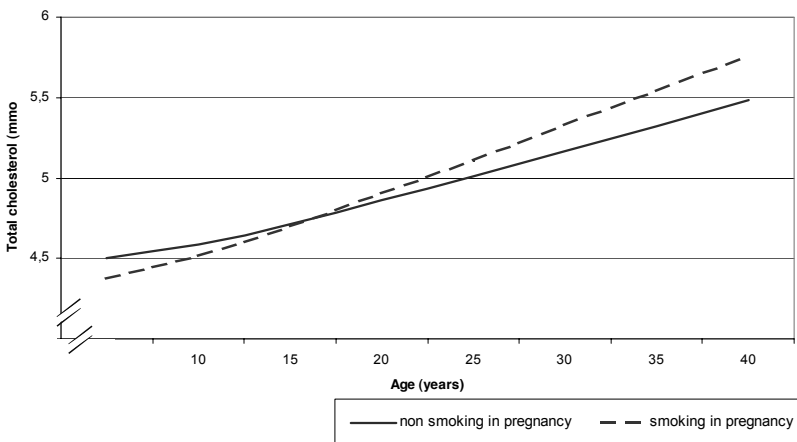
Figure 1. Maternal smoking in pregnancy and total blood cholesterol development in the offspring
Values are based on the following repeated measurements regression model:
Total cholesterol = $\beta_0 + \beta_1$*smoking in pregnancy + β_2*gender + β_3*age + β_4*age$^{-0.5}$ + β_5*smoking in pregnancy*age.

Table 1. General characteristics of men and women at their first and last visit

	Men (n = 192)	Women (n = 158)
Baseline (1975-1978)		
Age (years)	13.3 (4.2)	12.9 (4.0)
Height (cm)	157.6 (21.7)	152.3 (17.0)
Weight (kg)	47.2 (17.7)	44.2 (14.7)
Body mass index (kg/m²)	18.2 (2.7)	18.4 (3.1)
Total blood cholesterol (mmol/l)	4.6 (0.8)	4.7 (0.7)
Birth weight (grams)	3520 (643)	3335 (551)
Gestational age (weeks)	39.1 (1.6)	39.1 (1.5)
Maternal smoking in pregnancy (%)	19.3	22.2
Last visit		
Age (years)	34.5 (7.0)	33.8 (7.0)
Height (cm)	181.6 (7.3)	168.6 (7.5)
Weight (kg)	82.8 (14.2)	71.1(15.0)
Body mass index (kg/m²)	25.0 (3.7)	24.9 (4.7)
Total blood cholesterol (mmol/l)	5.2 (1.2)	5.0 (0.9)
LDL-cholesterol (mmol/l)	3.6 (1.1)	3.4 (1.0)
HDL-cholesterol (mmol/l)	1.1 (0.3)	1.4 (0.3)
Smoking (%)	42.7	30.9
Alcohol use (%)	86.5	81.0
Educational level (%)		
Elementary / secondary school	20.9	26.4
Lower / intermediate vocational training	54.0	51.8
Higher vocational training / university	25.1	21.8
Follow-up (years)*	23.7 (6.0 – 26.5)	23.4 (3.1 - 26.9)

Values are expressed as mean (standard deviation) in case of continuous variables and as percentages in case of categorical variables.
* Median (95% range)

Figure 1 shows the development of total cholesterol levels in the offspring from mothers who smoked and who did not smoke in pregnancy. Total cholesterol showed a higher annual rise (0.01 (95% CI: 0, 0.02) mmol/l per year) leading to increased total cholesterol levels from early adulthood onwards in the offspring of mothers who smoked in pregnancy compared to the offspring of mothers who did not smoke in pregnancy. Figure 2 shows that HDL-cholesterol and LDL-cholesterol tended towards a lower level (-0.05 (95% CI: -0.11, 0) mmol/l) and higher level (0.19 (95% CI: -0.05, 0.43) mmol/l), respectively, in the offspring of mothers who smoked in pregnancy than in the offspring of mothers who did not smoke in pregnancy.

Table 2 demonstrates the effects of potential confounders on the associations of maternal smoking in pregnancy with development of total cholesterol, HDL-cholesterol

Table 2. Total cholesterol, HDL-cholesterol and LDL-cholesterol levels in the offspring of mothers who smoked in pregnancy compared to the offspring of mothers who did not smoke in pregnancy

	Difference in annual change in total cholesterol (mmol/l/year)	Difference in HDL-cholesterol (mmol/l)	Difference in LDL-cholesterol (mmol/l)
Basic model			
Maternal smoking, age, gender	0.01 (0, 0.02)	-0.05 (-0.11, 0)	0.19 (-0.05, 0.43)
Adjustment for potential confounders			
Basic model			
+ current height	0.01 (0, 0.03)*	-0.06 (-0.12, -0.01)*	0.19 (-0.05, 0.43)
+ current weight	0.01 (0, 0.02)	-0.06 (-0.11, 0)*	0.16 (-0.08, 0.40)
+ current body mass index	0.01 (0, 0.02)	-0.05 (-0.10, 0)	0.12 (-0.12, 0.36)
+ current smoking	0.01 (0, 0.03)*	-0.05 (-0,10, 0)	0.18 (-0,07 0.43)
+ current number of cigarettes	0.02 (0, 0.03)*	-0.07 (-0.14, -0.01)*	0.17 (-0.10, 0.43)
+ current alcohol consumption	0.01 (0, 0.03)*	-0.06 (-0.11, 0)*	0.20 (-0.04, 0.44)
+ educational level	0.01 (0, 0.03)*	-0.06 (-0.12, 0)	0.09 (-0,20 0.38)
+ birth weight	0.01 (0, 0.02)*	-0.05 (-0.11, 0)	0.20 (-0.03, 0.44)

Values are regression coefficients (95% confidence interval) based on repeated measurements models reflecting the difference between cholesterol levels between the offspring of mothers who smoked in pregnancy and who did not smoke in pregnancy.
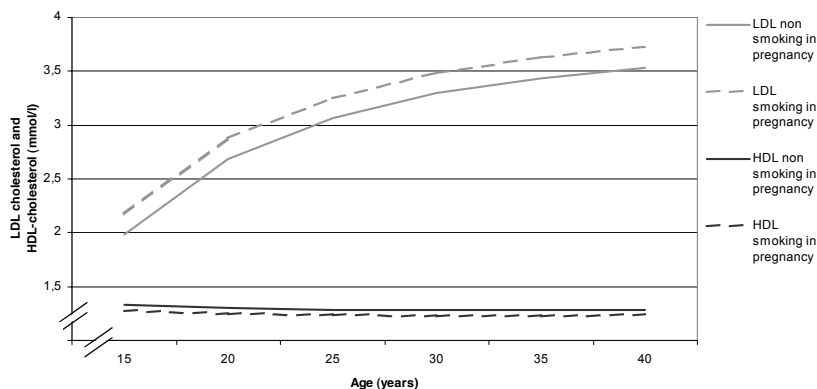*p-value < 0.05

Figure 2. Maternal smoking in pregnancy and LDL and HDL-cholesterol development in the offspring
Values are based on the following repeated measurements regression model:
HDL (LDL)-cholesterol = $\beta_0 + \beta_1$*smoking in pregnancy + β_2*gender + β_3*age + β_4*age$^{-0.5}$

and LDL-cholesterol levels in the offspring. The effect estimate of maternal smoking in pregnancy on total cholesterol was of borderline significance in the basic model including only age and gender as covariates. Adjustment for all covariates, except current weight and body mass index, revealed significant associations. The estimated effect of

maternal smoking in pregnancy on the longitudinal development of HDL-cholesterol and LDL-cholesterol was not materially affected by adjustment for potential confounders. Similar as for the total cholesterol models, the basic model, including age and gender, was of borderline significance for HDL-cholesterol. A statistically significant association appeared after adjustment for current height, current smoking habits and current alcohol consumption. In all models, LDL-cholesterol demonstrated tendencies towards higher levels in the offspring of mothers who smoked in pregnancy compared to the offspring of mothers who did not smoke in pregnancy. Birth weight did not materially change the effect estimates and was itself not associated with total cholesterol, HDL-cholesterol and LDL-cholesterol levels.

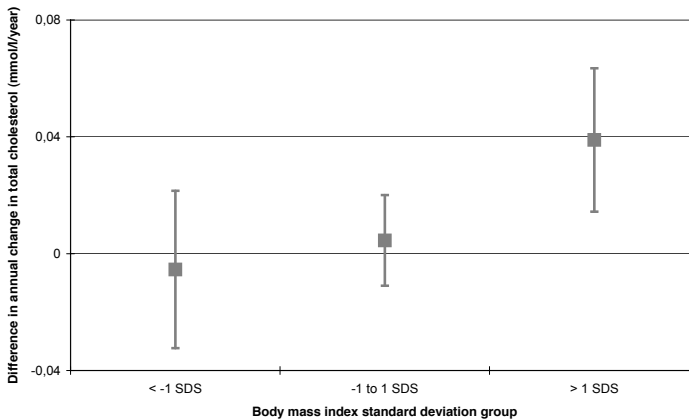Figure 3 shows the results of a stratified analysis within three body mass index

Figure 3. Difference in annual change in total blood cholesterol in the offspring of mothers who smoked in pregnancy compared to offspring of mothers who did not smoke in pregnancy within body mass index standard deviation score groups

groups, which revealed a stronger association within the highest group (body mass index standard deviation score > 1) There were no associations within the other body mass index standard deviation scores groups.

Discussion

This 27-year follow-up study demonstrates for the first time that maternal smoking in pregnancy is associated with an increased annual rise in total cholesterol levels from childhood into adulthood and trends towards an adverse lipoprotein profile in the off-

spring. The effect of maternal smoking in pregnancy on total cholesterol development was restricted to the offspring in the highest body mass index.

Strengths and limitations

The strength of this study is the long-term prospective follow-up with a large number of measurements performed in each subject. This large number increases the accuracy of the estimation of the true underlying cholesterol levels of each subject. A potential limitation of this study is that it was based on 61% of the subjects of the original cohort. Total cholesterol and body mass index at baseline were similar in those with and without information about maternal smoking in pregnancy. Loss to follow-up would lead to selection bias if the associations between maternal smoking in pregnancy and cholesterol level development differs between those lost to follow-up and those not lost to follow-up. This seems unlikely. Information about maternal smoking in pregnancy was retrospectively obtained by questionnaires sent to the mothers. Of the 353 completed and returned questionnaires, 99% (n = 350) contained information about maternal smoking in pregnancy. Although questionnaires seem to be a valid method for retrospective information collection on maternal smoking in pregnancy, misclassification may occur [15]. The mothers were not aware of the specific research question of this study at the moment of completing the questionnaires. Furthermore, the pregnancy data were obtained without reference to the cholesterol data and a large number of cholesterol measurements were conducted after returning the questionnaire about maternal smoking in pregnancy. Therefore, any misclassification is most likely to be non-differential and would tend to underestimate the effect size.

Maternal smoking and cholesterol development in the offspring

Maternal smoking in pregnancy was associated with an increased annual change in total cholesterol and trends towards adverse lipoprotein profiles in the offspring. These findings were independent of most potential confounding variables. Current body mass index was the only covariate that modified the estimated effect size. The effect of maternal smoking in pregnancy on total cholesterol development was restricted to the children with moderate overweight. Our findings are in line with studies that presented associations between low birth weight and subsequent increased cholesterol levels in childhood and adulthood. A recent systematic review of these studies suggested a decrease in total cholesterol of -0.04 (95% CI: -0.05, -0.03) mmol/l per kilogram increase in birth weight [4]. From a public health perspective, the authors concluded that low birth weight does not have a material impact on vascular risk in adulthood. However, the small effect sizes may be explained by misclassification due to measuring adverse fetal environment by only low birth weight. According to the fetal origins hypothesis, low birth weight is just a proxy for an adverse fetal environment. Direct measures of

an adverse fetal environment, including maternal smoking or maternal undernutrition, may reveal stronger associations with development of cardiovascular disease and its risk factors in the offspring.

Maternal smoking in pregnancy, which is the most important determinant of low birth weight in Western countries, may lead to an adverse fetal environment both by direct fetal exposure to nicotine and by fetal undernutrition due to associated dietary habits or placental dysfunction. Maternal smoking leads to a disproportional fetal growth retardation affecting primarily abdominal growth [20]. A small abdominal circumference may be a proxy for impaired hepatic growth, development and function including impaired lipid metabolism [21]. Maternal undernutrition or smoking in pregnancy may even induce developmental adaptations in organ development and function that are not accompanied by changes in growth characteristics. Experimental studies in rats suggested that protein restriction directly affects lipid metabolism and hepatic enzymes activity in the offspring without affecting growth [22, 23]. These results cannot easily be extrapolated to humans yet.

In our study, the associations of maternal smoking in pregnancy with cholesterol levels were not materially changed after adjustment for birth weight. This is in line with previous studies examining associations between maternal smoking in pregnancy and both obesity and increased blood pressure in the offspring [5, 6]. These findings suggest that the causal pathway from maternal smoking in pregnancy to development of cardiovascular risk factors in the offspring does not include birth weight per se. Fetal organ development may be permanently changed due to fetal exposure to nicotine despite only small changes in birth weight. Further studies examining the effect of maternal smoking in pregnancy on fetal and early postnatal organ growth and function could identify part of the underlying mechanisms.

Our findings are also in line with studies focused on maternal undernutrition instead of maternal smoking as measure of an adverse fetal environment. The Dutch Famine study found that individuals who were exposed in utero to maternal undernutrition had an increased risk of developing obesity and impaired glucose tolerance [16, 17]. These associations were independent of birth weight. In utero exposure to maternal undernutrition was not associated with total cholesterol, HDL-cholesterol and LDL-cholesterol [18]. These findings were not be replicated in the Leningrad Siege study, which also studied subjects who were exposed to a famine in utero [19].

Body mass index

We demonstrated in a stratified analysis that the effect of maternal smoking in pregnancy on total cholesterol development was restricted to the offspring with moderate overweight (body mass index standard deviation score > 1). Thus, children of mothers who smoked in pregnancy and who develop overweight themselves are especially at

risk of developing higher cholesterol levels. Cumulative and synergistic effects of maternal smoking and current obesity per se may explain these findings. However, current obesity may also represent other current life style habits including diet, which may be more important. Further studies are necessary to assess these cumulative or synergistic effects and the underlying biological mechanisms.

Study implications

Our findings may be important with regard to the early etiology of increased cholesterol levels and the subsequent risk of cardiovascular disease. The estimated additional annual change in offspring of mothers who smoked in pregnancy would lead to an increase in total cholesterol level of 0.30 mmol/l over a period of 30 years. This effect size suggests potential implications for public health on a population level. Therefore other studies, especially focused on the underlying mechanisms, are necessary.

References

1. Barker DJ. Fetal origins of coronary heart disease. BMJ 1995;311:171-4.
2. Bateson P, Barker D, Clutton-Brock T, Deb D, D'Udine B, Foley RA, et al. Developmental plasticity and human health. Nature 2004;430:419-21.
3. Huxley R, Neil A, Collins R. Unravelling the fetal origins hypothesis: is there really an inverse association between birthweight and subsequent blood pressure? Lancet 2002;360:659-65.
4. Huxley R, Owen CG, Whincup PH, Cook DG, Colman S, Collins R. Birth weight and subsequent cholesterol levels: exploration of the "fetal origins" hypothesis. JAMA 2004;292:2755-64.
5. von Kries R, Toschke AM, Koletzko B, Slikker W, Jr. Maternal smoking during pregnancy and childhood obesity. Am J Epidemiol 2002;156:954-61.
6. Lawlor DA, Najman JM, Sterne J, Williams GM, Ebrahim S, Davey Smith G. Associations of parental, birth, and early life characteristics with systolic blood pressure at 5 years of age: findings from the Mater-University study of pregnancy and its outcomes. Circulation 2004;110:2417-23.
7. Valkenburg HA, Hofman A, Klein F, Groustra FN. An epidemiological study of risk indicators for cardiovascular diseases (EPOZ). I. Blood pressure, serum cholesterol level, Quetelet-index and smoking habits in an open population aged 5 years and older. Ned Tijdschr Geneeskd 1980;124:183-9.
8. Uiterwaal CS, Witteman JC, de Bruijn AM, Hofman A, Grobbee DE. Families and natural history of lipids in childhood: an 18-year follow-up study. Am J Epidemiol 1997;145:777-85.
9. van Gent CM, van der Voort HA, de Bruyn AM, Klein F. Cholesterol determinations. A comparative study of methods with special reference to enzymatic procedures. Clin Chim Acta 1977;75:243-51.
10. Burstein M, Scholnick HR, Morfin R. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J Lipid Res 1970;11:583-95.
11. Grove TH. Effect of reagent pH on determination of high-density lipoprotein cholesterol by precipitation with sodium phosphotungstate-magnesium. Clin Chem 1979;25:560-4.
12. Abel LL, Levy BB, Brodie BB, Kendall FE. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. J Biol Chem 1952;195:357-66.
13. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. Int J Epidemiol 1999;28:964-74.
14. SAS/STAT User's Guide. Cary NSII eds.;1998.

15. Tomeo CA, Rich-Edwards JW, Michels KB, Berkey CS, Hunter DJ, Frazier AL, et al. Reproducibility and validity of maternal recall of pregnancy-related events. Epidemiology 1999;10:774-7.
16. Ravelli GP, Stein ZA, Susser MW. Obesity in young men after famine exposure in utero and early infancy. N Engl J Med 1976;295:349-53.
17. Ravelli AC, van der Meulen JH, Michels RP, Osmond C, Barker DJ, Hales CN, et al. Glucose tolerance in adults after prenatal exposure to famine. Lancet 1998;351:173-7.
18. Roseboom TJ, van der Meulen JH, Osmond C, Barker DJ, Ravelli AC, Bleker OP. Plasma lipid profiles in adults after prenatal exposure to the Dutch famine. Am J Clin Nutr 2000;72:1101-6.
19. Stanner SA, Bulmer K, Andres C, Lantseva OE, Borodina V, Poteen VV, et al. Does malnutrition in utero determine diabetes and coronary heart disease in adulthood? Results from the Leningrad siege study, a cross sectional study. BMJ 1997;315:1342-8.
20. Pringle PJ, Geary MP, Rodeck CH, Kingdom JC, Kayamba-Kay's S, Hindmarsh PC. The influence of cigarette smoking on antenatal growth, birth size, and the insulin-like growth factor axis. J Clin Endocrinol Metab 2005;90:2556-62.
21. Barker DJ, Martyn CN, Osmond C, Hales CN, Fall CH. Growth in utero and serum cholesterol concentrations in adult life. BMJ 1993;307:1524-7.
22. Lucas A, Baker BA, Desai M, Hales CN. Nutrition in pregnant or lactating rats programs lipid metabolism in the offspring. Br J Nutr 1996;76:605-12.
23. Desai M, Byrne CD, Meeran K, Martenz ND, Bloom SR, Hales CN. Regulation of hepatic enzymes and insulin levels in offspring of rat dams fed a reduced-protein diet. Am J Physiol 1997;273:G899-904.

Early life characteristics and arterial stiffness in adulthood

5.4

Abstract

Background: The associations of maternal smoking in pregnancy, low birth weight and preterm birth with blood pressure in later life may be explained by increased arterial stiffness. The aim of this study was to examine whether these early life factors are associated with measures of general and local arterial stiffness in adulthood.

Methods: Aortic pulse wave velocity and carotid distensibility were measured in 362 subjects aged 37.5 (SD 4.5) years participating in a cohort study with a 27-year follow-up. Information about maternal smoking in pregnancy, birth weight and gestational age was obtained through questionnaires sent to the parents.

Results: Maternal smoking in pregnancy and birth weight were, after adjustment for age, gender, mean arterial pressure and heart rate not associated with aortic pulse wave velocity and carotid distensibility. Gestational age was positively associated with aortic pulse wave velocity (0.12 (95% confidence interval: 0.01, 0.23) per week) but was not associated with carotid distensibility. Additional adjustment for potential confounders did not materially change the effect estimates and their confidence intervals.

Conclusion: Maternal smoking in pregnancy and birth weight were not associated with arterial stiffness in adulthood. We found a weak positive association between gestational age and arterial stiffness. The causal pathway underlying the associations of early life characteristics with blood pressure in later life probably includes other mechanisms than arterial stiffness.

Introduction

High blood pressure has at least part of its origins in early life. It is known that blood pressure tracks to some extent from childhood into adulthood [1]. More recent studies suggested that not only childhood characteristics but also fetal and birth characteristics are associated with blood pressure levels in childhood and adulthood. These characteristics include maternal smoking in pregnancy, low birth weight and preterm birth [2-4].

Increased arterial stiffness has been identified as a factor contributing to the development of hypertension and may be one of the mechanisms underlying the associations of early life characteristics with blood pressure in later life [5]. Martyn and Greenwald hypothesized that an adverse fetal environment leads to both fetal growth retardation and reduced elastin synthesis in the large arteries and subsequently to permanent stiffer arteries and increased blood pressure in later life [6]. This hypothesis was tested in a limited number of studies examining the associations of birth weight and gestational age with arterial stiffness in children and adults [7-17]. Results from these studies are not conclusive. To our knowledge, no studies have been conducted examining the associations between maternal smoking in pregnancy and arterial stiffness in the offspring. Maternal smoking in pregnancy is the most important determinant of low birth weight in Western countries and may be a direct measure of an adverse fetal environment. Fetal exposure to maternal smoking may lead to an adverse fetal environment and subsequently vascular developmental changes due to the direct effect of nicotine and the effect of associated maternal dietary and life style habits.

The aim of the present study was to examine the associations of maternal smoking in pregnancy, birth weight and gestational age with measures of arterial stiffness in adulthood.

Methods

Design and study population

All subjects aged 5 years and older living in two districts in Zoetermeer, a suburban town in the Netherlands, were invited to participate in the Epidemiological Preventive Organization Zoetermeer (EPOZ) Study between 1975 and 1978 [18]. This is a population-based study on risk indicators for chronic disease. Of the 5,670 eligible children aged 5-19 years, 4,649 (response 82%) were included. From this group, a random sample of 596 children was selected for an annual follow-up study on the natural history of cardiovascular risk factors and their determinants. Complete data were available for 425 fathers and 454 mothers at baseline. The children visited the research center annually in the same month of the year, preferably at the same time of the day, between 1975 and

1993. The response for these annual visits gradually declined to 81% (n = 483) in 1993. In 2002, all subjects were invited again for additional data collection, including arterial stiffness measurements. The response for this visit was 61% (n = 362).

Blood pressure and anthropometric measurements

Blood pressure was measured using a Hawksley random-zero sphygmomanometer (Lancing, Sussex) according to a standardized protocol on the left brachial artery of a sitting subject after a resting period of 15 minutes [19]. A cuff of 23 by 10 or 14 cm was used depending on the arm circumference. The mean of two consecutive measurements was used in the analyses. Height and weight were measured without shoes and heavy clothing and body mass index was calculated (weight/height^2 (kg/m^2)). Information on life style factors including smoking habits and alcohol consumption was obtained through questionnaires.

Pregnancy and birth data

A questionnaire to obtain pregnancy and birth data was sent to the parents of the children in 1993. This questionnaire included questions about maternal smoking in pregnancy (no, yes), birth weight (grams) and gestational age (weeks). Of the 483 subjects in the study in 1993, the addresses of 33 parents could not be found and 2 children had died during follow-up. Of the 448 questionnaires sent, 353 were completed and returned (response 79%). In 2002, missing pregnancy and birth data were completed in subjects participating in the measurements at the research center.

Arterial stiffness measurements

Aortic pulse wave velocity and carotid distensibility were used to measure general and local arterial stiffness respectively. Participants were instructed to refrain from smoking, drinking coffee and tea and taking pain-medication on the same day of the measurements, and from drinking alcohol on the day before the measurements.

Aortic pulse wave velocity, a measure of aortic stiffness, was assessed using an automated device (Complior, Colson, Garges-lès-Gonesse Cx, France) that computed the time delay between the rapid upstroke of the feet of simultaneously recorded pulse waves in the carotid and femoral artery [20, 21]. Distances from the carotid artery sampling site to the suprasternal notch and from the suprasternal notch to the femoral artery sampling site were measured using a pair of compasses. Pulse wave velocity was calculated as the ratio between the distance travelled by the pulse wave and the foot-to foot time delay and expressed as meters per second (m/s). The average of ten successive measurements was used in the analysis to cover a complete respiratory cycle. Pulse wave velocity increases with increasing arterial stiffness. Before measurement of pulse wave velocity, blood pressure measurement was measured twice with a sphygmomanometer

after five minutes of rest and the mean was used in the analyses. Mean arterial pressure was calculated by the following formula: diastolic blood pressure + 1/3 *(systolic blood pressure-diastolic blood pressure).

Carotid distensibility measures the change in arterial diameter due to change in arterial pressure over the cardiac cycle at one specific point in the arterial tree and decreases with increasing arterial stiffness [22, 23]. The vessel wall motion of the right common carotid artery was measured by means of a Duplex scanner (Ultramark IV, ATL, Bethel, Washington, USA) connected to a vessel wall movement detector system with the subject in supine position. After five minutes of rest, a region at 1,5 cm proximal to the origin of the bulb of the carotid artery was identified using B-mode ultrasonography. The displacement of the arterial walls was obtained by processing the radio frequency signals originating from two-selected sample volumes positioned over the anterior and posterior walls. The end-diastolic diameter (D), the absolute stroke change in diameter (ΔD) during systole and the relative stroke change in diameter (ΔD/D) were computed as the mean of four cardiac cycles of three successive recordings. During the carotid distensibility measurement, blood pressure was measured at the brachial artery twice with a Dinamap automated blood pressure recorder (Critikon, Tampa, Florida, USA). Pulse pressure (ΔP) was calculated as the difference between systolic and diastolic blood pressure. The cross-sectional arterial wall distensibility coefficient (DC) was calculated according to the following equation DC = (2ΔD/D)/ ΔP (10^{-3} /kPa) [24].

Data analysis

Associations of early life characteristics with aortic pulse wave velocity and carotid distensibility were assessed using multiple linear regression models. Early life characteristics include maternal smoking in pregnancy, birth weight and gestational age. The associations of early life characteristics with arterial stiffness measures were first adjusted for age and gender (Model A). This model was extended with major determinants of arterial stiffness including mean arterial pressure and heart rate (Model B). Subsequently, model B was adjusted for body mass index, current smoking and current alcohol consumption (Model C). Selection of these confounders was based on previous studies examining the associations of early life factors and arterial stiffness [7-17]. Further adjustment for weight, height and total cholesterol did not materially change the effect estimates and their confidence intervals. These models are therefore not presented separately. All measures of association are presented with their 95% confidence interval (CI). Statistical analyses were performed using the Statistical Package of Social Sciences version 11.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

General characteristics of the subjects participating in 2002 are presented in Table 1. The mean age was 37.5 (SD 4.49) years. Table 2 demonstrates the age and gender adjusted regression coefficients for all main exposures and covariates on both aortic pulse wave velocity and carotid distensibility. Table 3 and 4 present the effect estimates of the main determinants for aortic pulse wave velocity and carotid distensibility, respectively. After full adjustment, maternal smoking in pregnancy and birth weight were not associated with aortic pulse wave velocity and carotid distensibility. Gestational age was weakly positively associated with aortic pulse wave velocity (0.12 (95% CI: 0, 0.23) m/s per week) but not with carotid distensibility.

Table 1. General characteristics of men and women

	Men (n = 194)	Women (n = 168)
Characteristics in adulthood		
Age (years)	38.0 (4.4)	36.9 (4.5)
Height (cm)	182.2 (6.8)	168.9 (7.5)
Weight (kg)	85.2 (13.7)	72.8 (15.1)
Body mass index (kg/m²)	25.6 (3.6)	25.5 (4.7)
Systolic blood pressure (mmHg)	123.0 (12.8)	115.6 (12.0)
Diastolic blood pressure (mmHg)	82.5 (9.3)	76.5 (9.1)
Mean arterial pressure (mmHg)	96.0 (9.8)	89.5 (9.4)
Pulse pressure (mmHg)	40.5 (8.6)	39.1(8.0)
Heart rate (beats/min)	65.6 (11.2)	69.2 (10.9)
Aortic pulse wave velocity (m/s)	10.0 (1.5)	8.6 (1.2)
Carotid distensibility coefficient (10^{-3}/kPa)	23.3 (5.8)	27.0 (7.6)
Current smoking (%)	38.7	29.3
Current alcohol consumption (%)	86.4	77.4
Early life characteristics		
Maternal smoking in pregnancy (%)	14.2	21.9
Gestational age (weeks)	39.1 (1.5)	39.1 (1.5)
Birth weight (grams)	3491 (619)	3312 (533)

Values are expressed as mean (standard deviation) in case of continuous variables and as percentages in case of categorical variables.

Table 2. Univariate regression coefficients of subject characteristics on arterial stiffness measures

	Change per standard deviation or category increase	
	Aortic pulse wave velocity (m/s)	Carotid distensibility (10^{-3}/kPa)
Characteristics in adulthood		
Age (1 SD = 4.4 years)	0.41 (0.26, 0.56)**	-1.52 (-2.23, -0.81)**
Height (1 SD = 9.8 cm)	0.59 (0.44, 0.74)**	-1.40 (-2.12, -0.67)**
Weight (1SD = 15.6 kg)	0.38 (0.23, 0.54)**	-2.65 (-3.32, -1.98)**
Body mass index (1 SD = 4.2 kg/m^2)	0.06 (-0.11, 0.22)	-2.30 (-2.98, -1.62)**
Systolic blood pressure (1 SD = 13.0 mmHg)	0.53 (0.38, 0.69)**	-2.94 (-3.57, -2.30)**
Diastolic blood pressure (1 SD = 9.7 mmHg)	0.52 (0.37, 0.69)**	-2.68 (-3.33, -2.03)**
Mean arterial pressure (1SD = 10.1 mmHg)	0.56 (0.41, 071)**	-2.94 (-3.57, -2.30)**
Heart rate (1 SD = 11.2 beats/min)	0.24 (0.08, 0.39)**	-1.90 (-2.61, -1.20)**
Current smoking (difference yes / no)	-0.01 (-0.05, 0.04)	0.42 (-1.14, 1.98)
Current alcohol consumption (difference yes / no)	-0.07 (-0.16, 0.01)	-0.29 (-2.19, 1.62)
Early life characteristics		
Maternal smoking in pregnancy (difference yes / no)	-0.24 (-0.73 0.26)	0.94 (-1.29, 3.18)
Gestational age (1 SD = 1.49 weeks)	0.18 (-0.01, 0.37)	0.26 (-0.61, 1.12)
Birth weight (1SD = 585 grams)	0.20 (0.04, 0.37)**	-0.27 (-1.05, 0.51)

**p-value < 0.01

Table 3. Associations of early life characteristics with aortic pulse wave velocity

	Change in aortic pulse wave velocity (m/s) per 1 unit increase		
	Model A	Model B	Model C
Maternal smoking in pregnancy (difference yes / no)	-0.10 (-0.54, 0.33)	-0.29 (-0.74, 0.16)	-0.33 (-0.79, 0.14)
Gestational age (weeks)	0.13 (0.16, 0.24)*	0.12 (0.01, 0.23)*	0.12 (0.01, 0.23)*
Birth weight (kg)	0.19 (-0.07, 0.44)	0.17 (-0.09, 0.42)	0.16 (-0.10, 0.42)

Values are regression coefficients (95% confidence interval)
Model A: adjusted for age and gender.
Model B: adjusted for age, gender, mean arterial pressure and heart rate.
Model C: adjusted for age, gender, mean arterial pressure, heart rate, body mass index, smoking and alcohol consumption.
* p-value < 0.05

Table 4. Associations of early life characteristics with carotid distensibility

	Change in carotid distensibility (10^{-3}/kPa) per 1 unit increase		
	Model A	Model B	Model C
Maternal smoking in pregnancy (difference yes / no)	0.60 (-1.55, 2.76)	-0.05 (-2.08, 1.99)	-0.49 (-2.53, 1.55)
Gestational age (week)	0.17 (-0.39, 0.74)	0.21 (-0.30, 0.72)	0.20 (-0.30, 0.70)
Birth weight (kg)	-0.03 (-1.32, 1.26)	-0.26 (-1.41, 0.90)	-0.16 (-1.31, 0.99)

Values are regression coefficients (95% confidence interval)
Model A: adjusted for age and gender.
Model B: adjusted for age, gender, mean arterial pressure and heart rate.
Model C: adjusted for age, gender, mean arterial pressure, heart rate, body mass index, smoking and alcohol consumption.

Discussion

In this cohort study, we did not find associations of maternal smoking in pregnancy and birth weight with arterial stiffness in adulthood. We found a weak positive association between gestational age and aortic pulse wave velocity.

Methodological issues

A potential limitation of this study is that measurements of arterial stiffness were available in 61% of the subjects of the original cohort. Lost to follow-up would lead to selection bias if the associations of parental and early life characteristics with arterial stiffness differ between those lost to follow-up and those not lost to follow-up. This seems unlikely. Blood pressure and body mass index at baseline were similar in those lost to follow-up and those not lost to follow-up. Information on maternal smoking in pregnancy, birth weight and gestational age was retrospectively obtained by questionnaires sent to the mothers. Of all subjects participating in 2002 with arterial stiffness measurements, birth weight was known in 91% and gestational age and whether mother smoked in pregnancy was known in 70%. Although questionnaires seem to be a valid method for retrospective information collection on maternal smoking in pregnancy and birth outcomes, misclassification may be present [25]. However, the mothers were not aware of the specific research question at the moment of completing the questionnaires. Furthermore, the pregnancy and birth data were obtained without reference to the arterial stiffness measurements. Therefore, misclassification is most likely to be random and, if anything, would tend to underestimate the effect size.

Measurement of arterial stiffness

The main elastic materials in the arterial wall are collagen and elastin. The distribution of elastin and collagen in the arterial wall differs between the central and peripheral arter-

ies [6]. In the proximal aorta, elastin is the dominant component, in the distal aorta the content reverses and in the peripheral arteries collagen dominates. Since elastin is much more elastic than collagen, the arteries become stiffer with increasing distance from the heart. We used two methods for measuring arterial stiffness. Before interpreting our results, several methodological aspects of these methods need to be discussed.

Aortic pulse wave velocity measures arterial stiffness over a large part of the arterial tree thereby providing a measure of general arterial stiffness [20]. It combines properties of elastic arteries (proximal aorta) with more muscular arteries (distal aorta, iliac and femoral artery). This makes it difficult to evaluate differences in determinants of arterial stiffness between elastic and more muscular arteries. Using the distance between the carotid and femoral arteries leads to an overestimation of the real distance travelled by the pulse wave, resulting in higher mean values of aortic pulse wave velocity. Since variations in the anatomy are limited and this error may be considered similar for all subjects examined, it is unlikely that this limitation has seriously biased our results.

Carotid distensibility measures arterial stiffness at one site in the common carotid artery. An important limitation of measurements at one site is that this site may be not representative of a larger area of that artery and may be biased due to local vascular changes including atherosclerotic lesions. In computing the carotid distensibility coefficient we used the brachial artery pulse pressure and not the carotid pulse pressure. Information on comparisons between carotid and brachial artery pulse pressure indicates that the difference between these pressures is 8 mmHg in a presumed healthy population and 2.6 mmHg in patients with severe coronary heart disease [26]. Using brachial artery pulse pressure may have led to an underestimation of the distensibility coefficient. Since our study population was relatively young and healthy, the differences between brachial and carotid artery pulse pressure are expected to be relatively stable among subjects and are not likely to bias our results. The main advantage of the present study is that we used both aortic pulse wave velocity and carotid distensibility as measures of arterial stiffness.

The effect of early life factors on arterial stiffness

The main hypothesis for this study is that an adverse fetal environment leads to fetal growth retardation and a reduced elastin synthesis in the large arteries and subsequently to permanent stiffer arteries [6]. In our study, maternal smoking, low birth weight and preterm birth were used as measures of an adverse early environment and development.

Recent studies have suggested that maternal smoking in pregnancy is associated with increased blood pressure in the offspring in childhood [2]. Fetal exposure to maternal smoking may lead to an adverse fetal environment and subsequently vascular developmental changes due to the direct effect of nicotine and the effect of associ-

ated maternal dietary and life style habits. In adults, both active and passive smoking were associated with increased arterial stiffness, independent of blood pressure [27]. To our knowledge, the present study is the first exploring the association between maternal smoking in pregnancy and arterial stiffness in the offspring. Since we did not find associations of maternal smoking in pregnancy with aortic pulse wave velocity and carotid distensibility, the mechanisms underlying the associations of maternal smoking in pregnancy with increased blood pressure in the offspring may be others than arterial stiffness.

Birth weight was not associated with aortic pulse wave velocity and carotid distensibility. Other studies used only aortic pulse wave velocity or carotid distensibility measurements and demonstrated conflicting results but do not suggest systematic differences between results from studies with aortic pulse wave velocity or carotid distensibility as outcome [10-17]. These studies are difficult to compare with each other and with the present study because of the differences in methods used to assess arterial stiffness and differences in statistical adjustment. A potential explanation for previously demonstrated associations between small size at birth and increased arterial stiffness may, especially in middle-aged and older subjects, reflect other vascular processes including atherosclerosis. We found a weak positive association of gestational age with aortic pulse wave velocity but not with carotid distensibility. Recently, it has been suggested that also preterm birth is associated with arterial stiffness [14]. We are not aware of any previous studies demonstrating positive associations between gestational age and arterial stiffness and cannot explain this association. Further studies are necessary to elucidate the associations between gestational age and birth weight and arterial stiffness.

Conclusion

Maternal smoking in pregnancy and birth weight were not associated with arterial stiffness in adulthood. We found a weak positive association between gestational age and arterial stiffness. The causal pathway underlying the previously suggested associations of early life characteristics with increased blood pressure in later life probably includes other mechanisms than arterial stiffness.

References

1. Nelson MJ, Ragland DR, Syme SL. Longitudinal prediction of adult blood pressure from juvenile blood pressure levels. Am J Epidemiol 1992;136:633-45.
2. Lawlor DA, Najman JM, Sterne J, Williams GM, Ebrahim S, Davey Smith G. Associations of parental, birth, and early life characteristics with systolic blood pressure at 5 years of age: findings from the Mater-University study of pregnancy and its outcomes. Circulation 2004;110:2417-23.
3. Irving RJ, Belton NR, Elton RA, Walker BR. Adult cardiovascular risk factors in premature babies. Lancet 2000;355:2135-6.
4. Uiterwaal CS, Anthony S, Launer LJ, Witteman JC, Trouwborst AM, Hofman A, et al. Birth weight, growth, and blood pressure: an annual follow-up study of children aged 5 through 21 years. Hypertension 1997;30:267-71.
5. Liao D, Arnett DK, Tyroler HA, Riley WA, Chambless LE, Szklo M, et al. Arterial stiffness and the development of hypertension. The ARIC study. Hypertension 1999;34:201-6.
6. Martyn CN, Greenwald SE. Impaired synthesis of elastin in walls of aorta and large conduit arteries during early development as an initiating event in pathogenesis of systemic hypertension. Lancet 1997;350:953-5.
7. Cheung YF, Taylor MJ, Fisk NM, Redington AN, Gardiner HM. Fetal origins of reduced arterial distensibility in the donor twin in twin-twin transfusion syndrome. Lancet 2000;355:1157-8.
8. Gardiner HM, Taylor MJ, Karatza A, Vanderheyden T, Huber A, Greenwald SE, et al. Twin-twin transfusion syndrome: the influence of intrauterine laser photocoagulation on arterial distensibility in childhood. Circulation 2003;107:1906-11.
9. Cheung YF, Wong KY, Lam BC, Tsoi NS. Relation of arterial stiffness with gestational age and birth weight. Arch Dis Child 2004;89:217-21.
10. Martin H, Hu J, Gennser G, Norman M. Impaired endothelial function and increased carotid stiffness in 9-year-old children with low birthweight. Circulation 2000;102:2739-44.
11. Montgomery AA, Ben-Shlomo Y, McCarthy A, Davies D, Elwood P, Smith GD. Birth size and arterial compliance in young adults. Lancet 2000;355:2136-7.
12. Styczynski G, Abramczyk P, Szmigielski C, Placha G, Gaciong Z. Birth size and arterial compliance in young adults. Lancet 2000;356:855-6.
13. Murray LJ, Gallagher AM, Boreham CA, Savage M, Smith GD. Sex specific difference in the relation between birth weight and arterial compliance in young adults: The Young Hearts Project. J Epidemiol Community Health 2001;55:665-6.
14. Oren A, Vos LE, Bos WJ, Safar ME, Uiterwaal CS, Gorissen WH, et al. Gestational age and birth weight in relation to aortic stiffness in healthy young adults: two separate mechanisms? Am J Hypertens 2003;16:76-9.
15. te Velde SJ, Ferreira I, Twisk JW, Stehouwer CD, van Mechelen W, Kemper HC. Birthweight and arterial stiffness and blood pressure in adulthood: results from the Amsterdam Growth and Health Longitudinal Study. Int J Epidemiol 2004;33:154-61.
16. Martyn CN, Barker DJ, Jespersen S, Greenwald S, Osmond C, Berry C. Growth in utero, adult blood pressure, and arterial compliance. Br Heart J 1995;73:116-21.
17. Kumaran K, Fall CH, Martyn CN, Vijayakumar M, Stein C, Shier R. Blood pressure, arterial compliance, and left ventricular mass: no relation to small size at birth in south Indian adults. Heart 2000;83:272-7.
18. Valkenburg HA, Hofman A, Klein F, Groustra FN. An epidemiological study of risk indicators for cardiovascular diseases (EPOZ). I. Blood pressure, serum cholesterol level, Quetelet-index and smoking habits in an open population aged 5 years and older. Ned Tijdschr Geneeskd 1980;124:183-9.
19. Hofman A, Valkenburg HA. Determinants of change in blood pressure during childhood. Am J Epidemiol 1983;117:735-43.

20. Asmar R, Benetos A, Topouchian J, Laurent P, Pannier B, Brisac AM, et al. Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. Hypertension 1995;26:485-90.

21. van Popele NM. Causes and consequences of arterial stiffness, an epidemiological approach (thesis) Erasmus MC, Rotterdam; 2000.

22. Hoeks AP, Brands PJ, Smeets FA, Reneman RS. Assessment of the distensibility of superficial arteries. Ultrasound Med Biol 1990;16:121-8.

23. Kool MJ, van Merode T, Reneman RS, Hoeks AP, Struyker Boudier HA, Van Bortel LM. Evaluation of reproducibility of a vessel wall movement detector system for assessment of large artery properties. Cardiovasc Res 1994;28:610-4.

24. Reneman RS, van Merode T, Hick P, Muytjens AM, Hoeks AP. Age-related changes in carotid artery wall properties in men. Ultrasound Med Biol 1986;12:465-71.

25. Tomeo CA, Rich-Edwards JW, Michels KB, Berkey CS, Hunter DJ, Frazier AL, et al. Reproducibility and validity of maternal recall of pregnancy-related events. Epidemiology 1999;10:774-7.

26. Waddell TK, Dart AM, Medley TL, Cameron JD, Kingwell BA. Carotid pressure is a better predictor of coronary artery disease severity than brachial pressure. Hypertension 2001;38:927-31.

27. Stefanadis C, Vlachopoulos C, Tsiamis E, Diamantopoulos L, Toutouzas K, Giatrakos N, et al. Unfavorable effects of passive smoking on aortic function in men. Ann Intern Med 1998;128:426-34.

General discussion

6

Background

In the past two decades, epidemiological studies demonstrated associations of low birth weight with cardiovascular disease and its risk factors in later life [1-3]. The fetal origins hypothesis proposes that an adverse fetal environment leads to developmental adaptations which permanently program the fetus' structure, physiology and metabolism [4]. This programming would lead to fetal growth retardation and low birth weight and would be in favour of short-term survival. Long-term effects of this programming would be detrimental and lead to cardiovascular disease. Several alternative hypotheses focused on environmental and genetic mechanisms that may underlie these associations, have been proposed [5-8]. Based on these hypotheses, the search for the origins of cardiovascular disease has recently been extended from epidemiological studies in adults and children into new studies focused on fetal and early postnatal life.

Although birth weight is easily measured and available from obstetric records, it is not the best marker for an adverse fetal environment or exposure. The same birth weight may be the result of various fetal exposures and growth patterns. Therefore, birth weight is not likely to be the causal factor per se. Studies that directly examine the effects of fetal exposures on cardiovascular disease and its risk factors, irrespective of birth weight, are necessary. Maternal life style habits, including smoking in pregnancy, are the most important determinants of low birth weight in Western countries [9]. An adverse fetal environment due to maternal smoking may lead to developmental changes by the direct effects of nicotine and the associated maternal life style and dietary habits. Therefore, maternal smoking in pregnancy may be a better marker of an adverse fetal environment than low birth weight.

The general aim of studies presented in this thesis, was to identify pathways leading from adverse fetal exposures, including smoking and alcohol consumption, to suboptimal fetal growth patterns and subsequently risk factors for cardiovascular disease in later life. The main merits and shortcomings of these studies have been discussed in the previous chapters. This chapter provides a more general discussion of the main findings, considers general methodological issues and gives suggestions for further research.

Main findings

Determinants of fetal growth retardation and low birth weight

Studies examining determinants of fetal growth retardation and low birth weight were conducted in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood among 9,778 mothers and their children [10]. Our studies were focused on maternal smoking and alcohol consumption in pregnancy.

Active maternal smoking in pregnancy seems to be the most important modifiable risk factor for low birth weight in Western countries [11, 12]. Smoking in pregnancy leads to low birth weight by decreased fetal supplies of both nutrients and oxygen and subsequently fetal growth retardation [11, 12]. Recently, it was suggested that passive maternal smoking in pregnancy, defined as exposure to environmental tobacco smoke, is also associated with low birth weight [13]. We found that active and passive maternal smoking in pregnancy were associated with a lower birth weight in the offspring (chapter 4.1). Continued active maternal smoking after pregnancy was known was associated with an increased risk of low birth weight and preterm birth. The effect of smoking on birth weight was already present in the lowest smoking categories. Maternal smoking until pregnancy was known, was not adversely associated with weight or gestational age at birth. Completely quitting to smoke in early pregnancy, rather than reducing the number of actively smoked cigarettes during pregnancy, was associated with beneficial effects on birth weight.

Since low birth weight is only a proxy for fetal growth retardation, it is not the best marker for assessing the adverse effects of smoking in pregnancy on fetal growth and development. Fetal growth retardation may lead to normal birth weight if the fetus was actually supposed to grow on the upper percentiles based on the genetic growth potential. Previous studies suggested that maternal smoking in pregnancy is associated with impaired fetal growth from a gestational age of 20 weeks onwards [14-19]. However, these studies were conducted in small groups or in hospital-based populations and were not able adjust for all potential confounders. We found that that continued active maternal smoking in pregnancy was associated with reduced growth of fetal head circumference, abdominal circumference and femur length (chapter 4.2). These impaired fetal growth rates led to smaller femur length from mid-pregnancy onwards and smaller head circumference and abdominal circumference from late pregnancy. The earlier and larger effects on femur length suggest that maternal smoking in pregnancy affects primarily peripheral tissues. Maternal smoking until pregnancy was known and quitting thereafter, did not adversely affect fetal growth patterns.

Excessive alcohol consumption in pregnancy is associated with various pregnancy complications including low birth weight, preterm birth and congenital anomalies [20-22]. The effects of heavy alcohol consumption in pregnancy can not easily be extrapolated to lower levels of alcohol consumption. Previous studies examining the effects of low or moderate alcohol consumption in pregnancy on birth outcomes were inconsistent. Several studies found adverse effects, whereas others did not find any effect or even reported protective effects on weight and gestational age at birth [23-26]. These inconsistent results may be due to differences in study design and assessment of maternal alcohol consumption habits. Our study showed that maternal alcohol consumption of less than one drink per day was not associated with an increased risk of delivering a low

birth weight or preterm born infant. For alcohol consumption of one to three drinks per day, tendencies for an increased risk of low birth weight and preterm birth were found (chapter 4.3).

Development of risk factors for cardiovascular disease

Left ventricular hypertrophy is a strong and independent risk factor of cardiovascular morbidity and mortality [28]. Since the human heart has its highest growth rates in fetal and early postnatal life, an adverse environment in this period may affect left ventricular growth [29]. Previous studies did not examine the associations of fetal growth characteristics with postnatal left ventricular mass in healthy children. In a subgroup of the Generation R Study, we found positive associations of abdominal circumference in late pregnancy and birth weight with left ventricular mass and aortic root diameter in early infancy (chapter 5.1). These associations were independent of current weight and length and suggest that smaller fetal size in late pregnancy is associated with persistent smaller left ventricular mass and aortic root diameter in early infancy. From our study, it is not known whether and to what extend these cardiac changes persist in later life. We hypothesize that a relatively smaller left ventricle and aortic root diameter may lead to insufficient cardiac functioning for increasing metabolic demands in postnatal life. The heart may respond to these increased demands by growth and remodelling. Since the number of heart cells is largely established in fetal life, this remodelling would lead to adaptation and growth of existing cells. This process may be in favour of short-term cardiac functioning but may eventually lead to relative left ventricular dysfunction, increased left ventricular mass or even left ventricular hypertrophy. This hypothesis is supported by studies in children and adults. Recently, it was demonstrated that low birth weight was, independently of current weight and height, associated with persistent smaller total coronary heart diameter, aortic root diameter and left ventricular outflow tract diameter in children aged 9 years [30]. Increased growth rate and weight change, as occur in most low birth weight children, are associated with increased left ventricular mass [31, 32]. Studies in adults demonstrated that low weight in infancy was associated with increased left ventricular mass in adults [33, 34]. Follow-up studies are needed to test this hypothesis.

Long-term follow-up studies were performed in the Epidemiological Prevention Organization Zoetermeer (EPOZ) Study. The EPOZ Study is a population-based prospective cohort study in 596 children, initially aged 5 to 19 years. Current follow-up is 27 years. Recent studies suggested that maternal smoking in pregnancy is associated with increased blood pressure in the offspring [35-37]. These studies were conducted in children until the age of 9 years. We demonstrated in the EPOZ study, which comprises a 27-year follow-up, associations between maternal smoking in pregnancy and increased annual changes of systolic and diastolic blood pressure in the offspring (chapter 5.2).

This increase leads to higher systolic and diastolic blood pressure levels from early adulthood onwards. The associations were not explained by low birth weight, suggesting that other causal pathways may be involved. Mechanisms leading from smoking to higher blood pressure and atherosclerosis in adults include platelet aggregation, impaired lipoprotein metabolism, inflammatory responses and arterial stiffness [38-41]. These mechanisms may also be involved in the associations between maternal smoking in pregnancy and higher blood pressure in the offspring.

In the same study, we examined the associations of maternal smoking in pregnancy with total cholesterol, HDL-cholesterol and LDL-cholesterol development from childhood into adulthood. Our study demonstrated for the first time that maternal smoking in pregnancy is associated with an increased annual rise in total cholesterol levels from childhood into adulthood and tendencies towards an adverse lipoprotein profile in the offspring (chapter 5.3). The effect of maternal smoking in pregnancy on total cholesterol development was restricted to the offspring in the highest body mass index group. The associations between maternal smoking and cholesterol may be explained by the effects of maternal smoking in pregnancy on fetal abdominal growth. Smoking in pregnancy leads to disproportional fetal growth retardation [42]. A small abdominal circumference may be a proxy for impaired hepatic growth, development and function including impaired lipids metabolism [43]. Maternal undernutrition or smoking in pregnancy may even induce developmental adaptations in organ development and function that are not accompanied by changes in growth characteristics. Experimental studies in rats suggested direct persistent effects of protein restriction on lipid metabolism and hepatic enzymes activity in the offspring [44, 45]. These results cannot easily be extrapolated to humans yet.

Increased arterial stiffness has been identified as a factor contributing to the development of hypertension and may be one of the mechanisms underlying the associations of early life characteristics with blood pressure in later life [46]. Martyn and Greenwald hypothesized that an adverse fetal environment leads to both fetal growth retardation and reduced elastin synthesis in the large arteries and subsequently to permanent stiffer arteries and increased blood pressure in later life [47]. This hypothesis has been tested in a limited number of studies examining the associations of birth weight and gestational age with arterial stiffness in children and adults [48-58]. Results from these studies are not conclusive. In our study, we did not find associations of maternal smoking in pregnancy and birth weight with arterial stiffness in adulthood (chapter 5.4). We found a weak positive association between gestational age and arterial stiffness, measured by aortic pulse wave velocity. Our findings suggest that the causal pathway underlying the previously found associations of maternal smoking in pregnancy, low birth weight and preterm birth with increased blood pressure in later life, includes other mechanisms than arterial stiffness.

Methodological considerations

The methodological considerations of the studies presented in this thesis have been discussed in the separate chapters. In this paragraph, methodological issued regarding selection bias, information bias and confounding are discussed.

Selection bias

Our studies with fetal growth and birth weight as outcome were embedded in the Generation R Study. Of all eligible children at birth, 61% participate in this study. Among the participating mothers, information about smoking in pregnancy and alcohol consumption at enrolment was missing in 14%. Non-response due to non-participation and missing values at baseline among the participants is not likely to be random. National and regional registries do not have subject characteristics in all children and their parents that enable detailed non-response analyses. However, the percentages of mothers from ethnic minorities and lower socio-economic status and of mothers or children with medical complications are lower among the participants than expected from the population figures in Rotterdam [59]. Among the participating children, birth weight was 41 (95% confidence interval: 6, 76) grams lower when maternal information about smoking in pregnancy was missing. This selection towards a more affluent and healthy study population would lead to bias in our etiological association studies if the selection mechanisms are related to both the determinant and outcome and the associations differ between the study population and the eligible population. Although we do not expect that this is generally the case, the potential for selection bias is discussed in each chapter. The selection towards a more affluent and healthy study population affects probably the frequency rates and, as a consequence, the statistical power in our studies.

Selection bias in our studies embedded in the Generation R Study may not only be introduced due to selective non-response but also due to selective loss to follow-up. Major birth outcomes were available in 93% of all enrolled mothers and categories of maternal smoking habits at enrolment were similarly distributed among those lost to follow-up and those with singleton live birth as outcome. Lost to follow-up would lead to selection bias if the association of maternal smoking in pregnancy with fetal growth differs between those lost and those not lost to follow-up. This seems unlikely in our study.

In the EPOZ Study, the response at baseline was 82%. The population selected for follow-up in the EPOZ Study was a random sample of children, initially aged 5 to 19 years who participated in the baseline study. We do not believe that selection bias due to non-response has occurred since it is unlikely that children and their parents based their participation in the EPOZ Study on awareness of relations between early determi-

nants and later disease. To introduce selection bias, the association between early life characteristics and cardiovascular risk factors in adult life should differ between those participating and not participating.

A potential problem in this 27-year follow-up study is selective loss to follow-up leading to selection bias. However, our data do not indicate selective loss to follow-up. Baseline subject characteristics, including body mass index, blood pressure and total cholesterol were similar among those who were and who were not lost to follow-up. Furthermore, since the EPOZ Study comprises young adults, massive selective lost to follow-up due to manifest cardiovascular disease or other diseases, is not likely.

Information bias

Information about maternal smoking and alcohol consumption in pregnancy was obtained prospectively in the Generation R Study and retrospectively in the EPOZ Study by questionnaires sent to the mothers. This information was obtained without reference to fetal growth characteristics and cardiovascular risk factors. The mothers were not aware of the specific research questions addressed in this thesis. Although assessing life style habits in pregnancy by questionnaires seems to be a valid method, misclassification may occur [60]. This is especially the case for retrospective data collection as was conducted in the EPOZ Study.

Assessment of adverse life style habits by questionnaires may lead to underreporting. Random misclassification of the smoking and alcohol consumption categories would lead to bias towards the null. Alternatively, the estimated differences in fetal growth characteristics between the offspring of non-smoking and smoking mothers would be overestimated if underreporting would be selectively present among heavy smoking mothers who report low- to moderate smoking.

To overcome these limitations, other studies used biomarkers for smoking and alcohol consumption. Cotinine in maternal urine samples is used as biomarker for measuring tobacco exposure [61, 62]. However, low correlations between cotinine and self reported smoking habits have been demonstrated [63]. Possible explanations for these low correlations include inaccurate maternal reporting of smoking habits in pregnancy, use of categories of number of cigarettes smoked in questionnaires and individual differences in inhalation, absorption and metabolism. Previous studies demonstrated that using cotinine levels is not superior to self-report in studying the effect of maternal smoking in pregnancy on birth weight [64, 65]. Biomarkers of alcohol consumption including carbohydrate-deficient transferrin and gamma-glutamyl transferase, are not appropriate for assessment of light to moderate alcohol consumption because of their low sensitivity [66].

Confounding

Our main interest was in the effect of maternal smoking and alcohol consumption in pregnancy on fetal growth, birth weight and development of postnatal cardiovascular risk factors. Adjustment for demographic characteristics including maternal ethnicity, parity, educational level and age weakened the strengths of the associations. We used educational level as measure of socio-economic status in our models. Socio-economic status is strongly associated with birth weight [11]. Suggested biological pathways explaining associations between maternal socio-economic status and birth weight in the offspring include maternal smoking habits, body mass index, ethnicity and parity [67]. Studies identifying the biological mechanisms and their etiologic fractions explaining the associations of maternal socio-economic status and educational level in pregnancy with birth weight are necessary.

Future research

Our studies suggest that maternal smoking in pregnancy and reduced fetal growth affect postnatal development of cardiovascular risk factors. These findings underline the importance of focusing on exposures and growth patterns in fetal life rather than on birth weight in studying the fetal origins of cardiovascular disease.

Maternal smoking in pregnancy was related to reduced fetal growth, lower birth weight and postnatal development of cardiovascular risk factors. These results suggest that fetal exposure to smoking leads to adaptation mechanisms that predispose the individual to development of cardiovascular disease in adulthood. Further studies are needed to examine the direct effects of maternal smoking in pregnancy on these adaptation mechanisms, including changes in fetal organ development and to explore the causal pathways leading from maternal smoking in pregnancy to postnatal cardiovascular risk factors. Our finding of effect modification by postnatal body mass index of the association of maternal smoking in pregnancy with total cholesterol development, indicates the importance of including postnatal life style habits and growth patterns in future studies.

Well-designed epidemiological studies are necessary to overcome the existing methodological limitations, to identify the causal pathways and to quantify the effect sizes for public health. Such studies have recently been started or are currently planned [68-72]. The general approach of these studies is to assess prospectively environmental and genetic determinants of fetal and postnatal growth patterns and subsequently the development of risk factors for common diseases in childhood and adulthood. Information from these studies is needed to develop strategies for identifying groups at risk and prevention focused on the earliest phase of life. Obviously, in the meanwhile, public

health strategies for pregnant women should be aimed at non-smoking at all or quitting completely to smoke in early pregnancy.

References

1. Frankel S, Elwood P, Sweetnam P, Yarnell J, Smith GD. Birthweight, body-mass index in middle age, and incident coronary heart disease. Lancet 1996;348:1478-80.
2. Curhan GC, Willett WC, Rimm EB, Spiegelman D, Ascherio AL, Stampfer MJ. Birth weight and adult hypertension, diabetes mellitus, and obesity in US men. Circulation 1996;94:3246-50.
3. Rich-Edwards JW, Colditz GA, Stampfer MJ, Willett WC, Gillman MW, Hennekens CH, et al. Birthweight and the risk for type 2 diabetes mellitus in adult women. Ann Intern Med 1999;130:278-84.
4. Barker DJ. Fetal origins of coronary heart disease. BMJ 1995;311:171-4.
5. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardio-vascular disease in adult life. Lancet 1993;341:938-41.
6. Edwards CR, Benediktsson R, Lindsay RS, Seckl JR. Dysfunction of placental glucocorticoid barrier: link between fetal environment and adult hypertension? Lancet 1993;341:355-7.
7. Hattersley AT, Tooke JE. The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. Lancet 1999;353:1789-92.
8. Singhal A, Lucas A. Early origins of cardiovascular disease: is there a unifying hypothesis? Lancet 2004;363:1642-5.
9. Kramer MS, Olivier M, McLean FH, Dougherty GE, Willis DM, Usher RH. Determinants of fetal growth and body proportionality. Pediatrics 1990;86:18-26.
10. Hofman A, Jaddoe VW, Mackenbach JP, Moll HA, Snijders RF, Steegers EA, et al. Growth, development and health from early fetal life until young adulthood: the Generation R Study. Paediatr Perinat Epidemiol 2004;18:61-72.
11. Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. Bull World Health Organ 1987;65:663-737.
12. Abel EL. Smoking during pregnancy: a review of effects on growth and development of offspring. Hum Biol 1980;52:593-625.
13. Misra DP, Nguyen RH. Environmental tobacco smoke and low birth weight: a hazard in the work-place? Environ Health Perspect 1999;107:897-904.
14. Jeanty P, Cousaert E, de Maertelaer V, Cantraine F. Sonographic detection of smoking-related decreased fetal growth. J Ultrasound Med 1987;6:13-8.
15. Newnham JP, Patterson L, James I, Reid SE. Effects of maternal cigarette smoking on ultrasonic mea-surements of fetal growth and on Doppler flow velocity waveforms. Early Hum Dev 1990;24:23-36.
16. Vik T, Jacobsen G, Vatten L, Bakketeig LS. Pre- and post-natal growth in children of women who smoked in pregnancy. Early Hum Dev 1996;45:245-55.
17. Zaren B, Lindmark G, Bakketeig L. Maternal smoking affects fetal growth more in the male fetus. Paediatr Perinat Epidemiol 2000;14:118-26.
18. Bernstein IM, Plociennik K, Stahle S, Badger GJ, Secker-Walker R. Impact of maternal cigarette smok-ing on fetal growth and body composition. Am J Obstet Gynecol 2000;183:883-6.
19. Lampl M, Kuzawa CW, Jeanty P. Prenatal smoke exposure alters growth in limb proportions and head shape in the midgestation human fetus. Am J Hum Biol 2003;15:533-46.
20. Little RE. Moderate alcohol use during pregnancy and decreased infant birth weight. Am J Public Health 1977;67:1154-6.
21. Little RE, Wendt JK. The effects of maternal drinking in the reproductive period: an epidemiologic review. J Subst Abuse 1991;3:187-204.

22. Ouellette EM, Rosett HL, Rosman NP, Weiner L. Adverse effects on offspring of maternal alcohol abuse during pregnancy. N Engl J Med 1977;297:528-30.
23. Lazzaroni F, Bonassi S, Magnani M, Calvi A, Repetto E, Serra F, et al. Moderate maternal drinking and outcome of pregnancy. Eur J Epidemiol 1993;9:599-606.
24. Parazzini F, Chatenoud L, Surace M, Tozzi L, Salerio B, Bettoni G, et al. Moderate alcohol drinking and risk of preterm birth. Eur J Clin Nutr 2003;57:1345-9.
25. Lundsberg LS, Bracken MB, Saftlas AF. Low-to-moderate gestational alcohol use and intrauterine growth retardation, low birthweight, and preterm delivery. Ann Epidemiol 1997;7:498-508.
26. Whitehead N, Lipscomb L. Patterns of alcohol use before and during pregnancy and the risk of small-for-gestational-age birth. Am J Epidemiol 2003;158:654-62.
27. Valkenburg HA, Hofman A, Klein F, Groustra FN. An epidemiological study of risk indicators for cardiovascular diseases (EPOZ). I. Blood pressure, serum cholesterol level, Quetelet-index and smoking habits in an open population aged 5 years and older. Ned Tijdschr Geneeskd 1980;124:183-9.
28. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. N Engl J Med 1990;322:1561-6.
29. Ishii A, Tatsunami S, Satoh I, Honma T, Hamada H, Yago N. Growth dynamics of the heart from perinatal period to childhood. J Perinat Med 1990;18:459-63.
30. Jiang B, Godfrey KM, Martyn CN, Gale CR. Birth weight and cardiac structure in children. Pediatrics 2006;117:e257-61.
31. Urbina EM, Gidding SS, Bao W, Pickoff AS, Berdusis K, Berenson GS. Effect of body size, ponderosity, and blood pressure on left ventricular growth in children and young adults in the Bogalusa Heart Study. Circulation 1995;91:2400-6.
32. Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL. Children born small for gestational age: do they catch up? Pediatr Res 1995;38:267-71.
33. Vijayakumar M, Fall CH, Osmond C, Barker DJ. Birth weight, weight at one year, and left ventricular mass in adult life. Br Heart J 1995;73:363-7.
34. Zureik M, Bonithon-Kopp C, Lecomte E, Siest G, Ducimetiere P. Weights at birth and in early infancy, systolic pressure, and left ventricular structure in subjects aged 8 to 24 years. Hypertension 1996;27:339-45.
35. Morley R, Leeson Payne C, Lister G, Lucas A. Maternal smoking and blood pressure in 7.5 to 8 year old offspring. Arch Dis Child 1995;72:120-4.
36. Blake KV, Gurrin LC, Evans SF, Beilin LJ, Landau LI, Stanley FJ, et al. Maternal cigarette smoking during pregnancy, low birth weight and subsequent blood pressure in early childhood. Early Hum Dev 2000;57:137-47.
37. Lawlor DA, Najman JM, Sterne J, Williams GM, Ebrahim S, Davey Smith G. Associations of parental, birth, and early life characteristics with systolic blood pressure at 5 years of age: findings from the Mater-University study of pregnancy and its outcomes. Circulation 2004;110:2417-23.
38. Fusegawa Y, Goto S, Handa S, Kawada T, Ando Y. Platelet spontaneous aggregation in platelet-rich plasma is increased in habitual smokers. Thromb Res 1999;93:271-8.
39. Muscat JE, Harris RE, Haley NJ, Wynder EL. Cigarette smoking and plasma cholesterol. Am Heart J 1991;121:141-7.
40. Bazzano LA, He J, Muntner P, Vupputuri S, Whelton PK. Relationship between cigarette smoking and novel risk factors for cardiovascular disease in the United States. Ann Intern Med 2003;138:891-7.
41. Stefanadis C, Tsiamis E, Vlachopoulos C, Stratos C, Toutouzas K, Pitsavos C, et al. Unfavorable effect of smoking on the elastic properties of the human aorta. Circulation 1997;95:31-8.
42. Pringle PJ, Geary MP, Rodeck CH, Kingdom JC, Kayamba-Kay's S, Hindmarsh PC. The influence of cigarette smoking on antenatal growth, birth size, and the insulin-like growth factor axis. J Clin Endocrinol Metab 2005;90:2556-62.

43. Barker DJ, Martyn CN, Osmond C, Hales CN, Fall CH. Growth in utero and serum cholesterol concentrations in adult life. BMJ 1993;307:1524-7.

44. Lucas A, Baker BA, Desai M, Hales CN. Nutrition in pregnant or lactating rats programs lipid metabolism in the offspring. Br J Nutr 1996;76:605-12.

45. Desai M, Byrne CD, Meeran K, Martenz ND, Bloom SR, Hales CN. Regulation of hepatic enzymes and insulin levels in offspring of rat dams fed a reduced-protein diet. Am J Physiol 1997;273:G899-904.

46. Liao D, Arnett DK, Tyroler HA, Riley WA, Chambless LE, Szklo M, et al. Arterial stiffness and the development of hypertension. The ARIC study. Hypertension 1999;34:201-6.

47. Martyn CN, Greenwald SE. Impaired synthesis of elastin in walls of aorta and large conduit arteries during early development as an initiating event in pathogenesis of systemic hypertension. Lancet 1997;350:953-5.

48. Cheung YF, Taylor MJ, Fisk NM, Redington AN, Gardiner HM. Fetal origins of reduced arterial distensibility in the donor twin in twin-twin transfusion syndrome. Lancet 2000;355:1157-8.

49. Gardiner HM, Taylor MJ, Karatza A, Vanderheyden T, Huber A, Greenwald SE, et al. Twin-twin transfusion syndrome: the influence of intrauterine laser photocoagulation on arterial distensibility in childhood. Circulation 2003;107:1906-11.

50. Cheung YF, Wong KY, Lam BC, Tsoi NS. Relation of arterial stiffness with gestational age and birth weight. Arch Dis Child 2004;89:217-21.

51. Martin H, Hu J, Gennser G, Norman M. Impaired endothelial function and increased carotid stiffness in 9-year-old children with low birthweight. Circulation 2000;102:2739-44.

52. Montgomery AA, Ben-Shlomo Y, McCarthy A, Davies D, Elwood P, Smith GD. Birth size and arterial compliance in young adults. Lancet 2000;355:2136-7.

53. Styczynski G, Abramczyk P, Szmigielski C, Placha G, Gaciong Z. Birth size and arterial compliance in young adults. Lancet 2000;356:855-6.

54. Murray LJ, Gallagher AM, Boreham CA, Savage M, Smith GD. Sex specific difference in the relation between birth weight and arterial compliance in young adults: The Young Hearts Project. J Epidemiol Community Health 2001;55:665-6.

55. Oren A, Vos LE, Bos WJ, Safar ME, Uiterwaal CS, Gorissen WH, et al. Gestational age and birth weight in relation to aortic stiffness in healthy young adults: two separate mechanisms? Am J Hypertens 2003;16:76-9.

56. te Velde SJ, Ferreira I, Twisk JW, Stehouwer CD, van Mechelen W, Kemper HC. Birthweight and arterial stiffness and blood pressure in adulthood: results from the Amsterdam Growth and Health Longitudinal Study. Int J Epidemiol 2004;33:154-61.

57. Martyn CN, Barker DJ, Jespersen S, Greenwald S, Osmond C, Berry C. Growth in utero, adult blood pressure, and arterial compliance. Br Heart J 1995;73:116-21.

58. Kumaran K, Fall CH, Martyn CN, Vijayakumar M, Stein C, Shier R. Blood pressure, arterial compliance, and left ventricular mass: no relation to small size at birth in south Indian adults. Heart 2000;83:272-7.

59. Center for Research and Statistics, Rotterdam (COS); http:// www.cos.rotterdam.nl. In; 2005.

60. Klebanoff MA, Levine RJ, Morris CD, Hauth JC, Sibai BM, Ben Curet L, et al. Accuracy of self-reported cigarette smoking among pregnant women in the 1990s. Paediatr Perinat Epidemiol 2001;15:140-3.

61. Hebel JR, Fox NL, Sexton M. Dose-response of birth weight to various measures of maternal smoking during pregnancy. J Clin Epidemiol 1988;41:483-9.

62. Wang X, Tager IB, Van Vunakis H, Speizer FE, Hanrahan JP. Maternal smoking during pregnancy, urine cotinine concentrations, and birth outcomes. A prospective cohort study. Int J Epidemiol 1997;26:978-88.

63. English PB, Eskenazi B, Christianson RE. Black-white differences in serum cotinine levels among pregnant women and subsequent effects on infant birthweight. Am J Public Health 1994;84:1439-43.

64. England LJ, Kendrick JS, Gargiullo PM, Zahniser SC, Hannon WH. Measures of maternal tobacco exposure and infant birth weight at term. Am J Epidemiol 2001;153:954-60.

65. Haddow JE, Knight GJ, Palomaki GE, Kloza EM, Wald NJ. Cigarette consumption and serum cotinine in relation to birthweight. Br J Obstet Gynaecol 1987;94:678-81.

66. van Pelt J, Leusink GL, van Nierop PW, Keyzer JJ. Test characteristics of carbohydrate-deficient transferrin and gamma-glutamyltransferase in alcohol-using perimenopausal women. Alcohol Clin Exp Res 2000;24:176-9.

67. Kramer MS. Socioeconomic determinants of intrauterine growth retardation. Eur J Clin Nutr 1998;52 :S29-32.

68. Olsen J, Melbye M, Olsen SF, Sorensen TI, Aaby P, Andersen AM, et al. The Danish National Birth Cohort: its background, structure and aim. Scand J Public Health 2001;29:300-7.

69. Smith K, Joshi H. The Millennium Cohort Study. Popul Trends 2002:30-4.

70. Branum AM, Collman GW, Correa A, Keim SA, Kessel W, Kimmel CA, et al. The National Children's Study of environmental effects on child health and development. Environ Health Perspect 2003;111:642-6.

71. The Norwegian mother and child cohort study; http://www.fhi.no ; 2005.

72. Inskip HM, Godfrey KM, Robinson SM, Law CM, Barker DJ, Cooper C. Cohort profile: The Southampton Women's Survey. Int J Epidemiol 2006;35:42-8.

Summary

7

In the past two decades, epidemiological studies demonstrated associations of low birth weight with cardiovascular disease and its risk factors in later life. The fetal origins hypothesis proposes that an adverse fetal environment leads to developmental adaptations that permanently program the fetus' structure, physiology and metabolism. This programming would lead to fetal growth retardation and low birth weight and would be in favour of short-term survival. Long-term effects of this programming would be detrimental and lead to cardiovascular disease. Based on this hypothesis, the search for the origins of cardiovascular disease has recently been extended from epidemiological studies in adults and children into new studies focused on fetal and early postnatal life.

Although birth weight is easily measured and available from obstetric records, it is not likely to be the best marker for an adverse fetal environment or exposure. The same birth weight may be the result of various fetal exposures and growth patterns. Maternal smoking in pregnancy is the most important determinant of low birth weight in Western countries. An adverse fetal environment due to maternal smoking may lead to developmental changes by the direct effects of nicotine and the associated maternal life style and dietary habits. Therefore, maternal smoking in pregnancy may be a better marker of an adverse fetal environment than low birth weight.

The fetal origins hypothesis was the main point of departure for studies presented in this thesis. The general aim of these studies was to identify pathways leading from adverse fetal exposures to suboptimal fetal growth patterns and subsequently risk factors for cardiovascular disease in later life. The fetal exposure of main interest was maternal smoking in pregnancy.

Several hypotheses focused on mechanisms underlying the associations of low birth weight with diseases in adulthood have been proposed. These hypotheses proposed central roles for 1) fetal undernutrition; 2) increased fetal cortisol exposure; 3) genetic susceptibility to both low birth weight and diseases in adulthood; and 4) accelerated postnatal growth in low birth weight children. In **chapter 2**, epidemiological studies designed to explore these hypotheses, are reviewed. Thus far, it is not known which mechanisms underlie the associations of low birth weight with adult diseases. The causal pathways linking low birth weight to diseases in adulthood seem to be complex and may include combined environmental and genetic mechanisms in various periods of life. Well-designed epidemiological studies are necessary to identify the underlying mechanisms and to estimate the population effect size.

In **chapter 3**, the Generation R Study is presented. The Generation R Study is a population-based prospective cohort study from fetal life until young adulthood. The study is designed to identify early environmental and genetic causes of normal and abnormal growth, development and health from fetal life until young adulthood. The study focuses

on four primary areas of research: 1) growth and physical development; 2) behavioural and cognitive development; 3) diseases in childhood; and 4) health and healthcare for pregnant women and children. In total, 9,778 mothers with a delivery date from April 2002 until January 2006 were enrolled in the study. Of all eligible children at birth, 61% participate in the study. Data collection in the prenatal phase was planned in early pregnancy (gestational age < 18 weeks), mid-pregnancy (18 - 25 weeks) and late pregnancy (gestational age ≥ 25 weeks) and included physical examinations, questionnaires, fetal ultrasound examinations and biological samples. The children form a prenatally recruited birth-cohort that is currently followed until young adulthood.

Studies in **chapter 4** deal with the effect of maternal smoking and alcohol consumption in pregnancy on fetal growth and birth weight and were conducted in the Generation R Study.

Smoking in pregnancy leads to low birth weight by decreased fetal supplies of both nutrients and oxygen and subsequently fetal growth retardation. Recently, it was suggested that passive maternal smoking in pregnancy, defined as exposure to environmental tobacco smoke, is also associated with low birth weight. In the study presented in **chapter 4.1**, we found that active and passive maternal smoking in pregnancy were associated with a lower birth weight in the offspring. Continued active maternal smoking after pregnancy was known, was associated with an increased risk of low birth weight and preterm birth. The effect of smoking on birth weight was already present in the lowest smoking categories. Maternal smoking until pregnancy was known, was not adversely associated with weight or gestational age at birth. Completely quitting to smoke in early pregnancy, rather than reducing the number of actively smoked cigarettes during pregnancy, was associated with beneficial effects on birth weight in the offspring.

Since low birth weight is only a proxy for fetal growth retardation, it is not the best marker for assessing the adverse effects of smoking in pregnancy on fetal growth and development. Fetal growth retardation may lead to normal birth weight if the fetus was actually supposed to grow on the upper percentiles based on the genetic growth potential. We demonstrated in **chapter 4.2** that continued active maternal smoking in pregnancy was associated with reduced growth of fetal head circumference, abdominal circumference and femur length. These impaired fetal growth rates led to smaller femur length from mid-pregnancy onwards and smaller head circumference and abdominal circumference from late pregnancy. The earlier and larger effects on femur length suggest that maternal smoking in pregnancy affects primarily peripheral tissues. Maternal smoking until pregnancy was known and quitting thereafter did not adversely affect fetal growth patterns.

Excessive alcohol consumption in pregnancy is associated with various pregnancy complications including low birth weight, preterm birth and congenital anomalies. The effects of heavy alcohol consumption in pregnancy can not easily be extrapolated to lower levels of alcohol consumption. Our study, presented in **chapter 4.3** showed that maternal alcohol consumption of less than one drink per day was not associated with an increased risk of delivering a low birth weight or preterm born infant. For alcohol consumption of one to three drinks per day, tendencies for an increased risk of low birth weight and preterm birth were found.

Studies in **chapter 5** deal with the effects of fetal growth retardation and maternal smoking in pregnancy on the postnatal development of cardiovascular risk factors.

Left ventricular hypertrophy is a strong and independent risk factor of cardiovascular morbidity and mortality. Since the human heart has its highest growth rates in fetal and early postnatal life, an adverse environment in this period may affect left ventricular growth. The study presented in **chapter 5.1** was performed in a subgroup of the Generation R Study and showed positive associations of abdominal circumference in late pregnancy and birth weight with left ventricular mass and aortic root diameter in early infancy. These associations were independent of current weight and length and suggest that smaller fetal size in late pregnancy is associated with persistent smaller left ventricular mass and aortic root diameter in early infancy. Further studies are needed to assess whether and to what extend these cardiac changes persist in later life.

Long-term follow-up studies were performed in the Epidemiological Prevention Organization Zoetermeer (EPOZ) Study. The EPOZ Study is a population-based prospective cohort study in 596 children, initially aged 5 to 19 years. Current follow-up is 27 years.

Previous studies suggested that maternal smoking in pregnancy is associated with increased blood pressure in the offspring. These studies were conducted in children until the age of 9 years. The study presented in **chapter 5.2** was conducted in the EPOZ Study and showed that maternal smoking in pregnancy was associated with increased annual changes of systolic and diastolic blood pressure in the offspring. This increase led to higher systolic and diastolic blood pressure levels from early adulthood. The associations were not explained by low birth weight, suggesting that other causal pathways may be involved.

In the same study, we examined the associations of maternal smoking in pregnancy with total cholesterol, HDL-cholesterol and LDL-cholesterol development from childhood into adulthood. Our study, presented in **chapter 5.3**, demonstrated for the first time that maternal smoking in pregnancy was associated with an increased annual rise in total cholesterol levels from childhood into adulthood and tendencies towards an adverse lipoprotein profile in the offspring. The effect of maternal smoking in pregnancy

on total cholesterol development was restricted to the offspring in the highest body mass index group.

Increased arterial stiffness has been identified as a factor contributing to the development of hypertension and may be one of the mechanisms underlying the associations of early life characteristics with blood pressure in later life. Previously, it was hypothesized that an adverse fetal environment leads to both fetal growth retardation and reduced elastin synthesis in the large arteries and subsequently to permanent stiffer arteries and increased blood pressure in later life. This hypothesis was tested in the study presented in **chapter 5.4**. Results from this study suggest that the causal pathway underlying the previously found associations of maternal smoking in pregnancy, low birth weight and preterm birth with increased blood pressure in later life, includes other mechanisms than arterial stiffness.

In **chapter 6** the general discussion, the results described in this thesis are considered in a broader context. In addition, relevant methodological issues are discussed and suggestions for future research are given.

Samenvatting

In de afgelopen twintig jaar zijn in epidemiologisch onderzoek associaties aangetoond tussen een laag geboortegewicht en de ontwikkeling van hart en vaatziekten en de risicofactoren daarvoor op latere leeftijd. De 'foetale origine van volwassen aandoeningen' hypothese veronderstelt dat een ongunstige foetale omgeving leidt tot aanpassingen in de ontwikkeling die permanent de structuur, fysiologie en het metabolisme van de foetus beïnvloeden. Dit leidt tot foetale groeivertraging en een laag geboortegewicht en zou ten gunste zijn voor de overleving op korte termijn. Lange termijn effecten zouden echter schadelijk zijn en leiden tot hart en vaatziekten. Door deze hypothese is recent de zoektocht naar de oorsprong van hart en vaatziekten uitgebreid van epidemiologisch onderzoek bij volwassenen en kinderen naar onderzoek gericht op het foetale en vroege postnatale leven.

Hoewel het geboortegewicht makkelijk te meten is en beschikbaar is uit obstetrische dossiers, is het waarschijnlijk niet de beste afspiegeling van een ongunstige foetale omgeving of blootstelling. Hetzelfde geboortegewicht kan het resultaat zijn van verschillende foetale blootstellingen en groeipatronen. Roken van moeder tijdens de zwangerschap is de belangrijkste determinant van laag geboortegewicht in westerse landen. Een ongunstige foetale omgeving als gevolg van roken van moeder kan door de directe effecten van nicotine en de geassocieerde maternale levensstijl en voedingsgewoonten leiden tot veranderingen in de ontwikkeling. Om die reden zou roken van moeder tijdens de zwangerschap een betere afspiegeling kunnen zijn van een nadelige foetale omgeving dan het geboortegewicht.

De 'foetale origine van volwassen aandoeningen' hypothese was de belangrijkste aanleiding tot het doen van het onderzoek dat beschreven wordt in dit proefschrift. Het doel van dit onderzoek was om mechanismen te identificeren die leiden van ongunstige foetale blootstellingen, tot suboptimale foetale groeipatronen en vervolgens tot de ontwikkeling van risicofactoren voor hart en vaatziekten. Hierbij hebben we ons gericht op roken van moeder tijdens de zwangerschap als ongunstige foetale blootstelling.

Er zijn verschillende hypothesen voorgesteld voor mechanismen die de associaties tussen een laag geboortegewicht en ziekten op de volwassen leeftijd zouden kunnen verklaren. Deze hypothesen stellen een centrale rol voor voor 1) foetale ondervoeding; 2) toegenomen foetale blootstelling aan cortisol; 3) genetische aanleg voor zowel laag geboortegewicht als ziekten op de volwassen leeftijd; en 4) versnelde postnatale groei van kinderen met een laag geboortegewicht. In **hoofdstuk 2** worden resultaten beschreven van eerder verricht epidemiologisch onderzoek, dat opgezet was om deze hypothesen te testen. Het is nog niet bekend welke mechanismen de associaties tussen laag geboortegewicht en ziekten op de volwassen leeftijd verklaren. De mechanismen

die leiden van een laag geboortegewicht tot ziekten op latere leeftijd lijken complex te zijn en gecombineerde omgevings- en genetische factoren in verschillende perioden van het leven te omvatten. Goed opgezet epidemiologisch onderzoek is nodig om de verklarende mechanismen te identificeren en om de grote van het effect op populatie-niveau te schatten.

In **hoofdstuk 3** wordt het Generation R onderzoek gepresenteerd. Het Generation R onderzoek, is een populatiegebaseerd prospectief cohort onderzoek vanaf het vroe-ge foetale leven tot de jongvolwassenheid. Het onderzoek is opgezet om de vroege omgevings- en genetische oorzaken van normale en abnormale groei, ontwikkeling en gezondheid van het foetale leven tot aan de jongvolwassenheid te identificeren. Het onderzoek is gericht op vier primaire onderzoeksgebieden: 1) groei en ontwikkeling; 2) gedrag en cognitieve ontwikkeling; 3) ziekten op de kinderleeftijd en 4) zorg en zorg-gebruik voor zwangere vrouwen en kinderen. In totaal zijn 9.778 moeders geïncludeerd met een bevallingsdatum tussen april 2002 en januari 2006. Van alle kinderen die bij de geboorte in aanmerking kwamen, doet 61% mee aan het onderzoek. Data verzameling in de prenatale fase was gepland in de vroege zwangerschap (zwangerschapsduur < 18 weken), halverwege de zwangerschap (zwangerschapsduur 18-25 weken) en laat in de zwangerschap (zwangerschapsduur ≥ 25 weken) en omvatte lichamelijk onderzoek, vragenlijsten, foetaal echo-onderzoek en biologische monsters. De kinderen vormen een prenataal geïncludeerd geboortecohort dat gevolgd wordt tot aan de jongvolwas-senheid.

Onderzoek beschreven in **hoofdstuk 4** is gericht op het effect van roken en alcoholge-bruik van moeder tijdens de zwangerschap op de foetale groei en het geboortegewicht. Dit onderzoek werd uitgevoerd in het Generation R onderzoek.

Roken tijdens de zwangerschap leidt tot een laag geboortegewicht door vermin-derde foetale voorraden van zowel voedingsstoffen als zuurstof en de daaruitvolgende groeivertraging. Recent werd gesuggereerd dat ook passief roken van moeder tijdens de zwangerschap, gedefinieerd als blootstelling aan tabaksrook in de omgeving, geas-socieerd is met een laag geboortegewicht. In het onderzoek gepresenteerd in **hoofd-stuk 4.1** vonden we dat actief en passief roken van moeder geassocieerd waren met een laag geboortegewicht. Actief roken van moeder nadat de zwangerschap bekend was, was ook geassocieerd met een toegenomen risico op vroeggeboorte. De effecten van roken op het geboortegewicht waren al te zien in de minst blootgestelde groepen. Roken van moeder tot de zwangerschap bekend was, was niet nadelig geassocieerd met het gewicht of de zwangerschapsduur bij de geboorte. Volledig stoppen met roken vroeg in de zwangerschap was, meer dan het verminderen van het aantal actief

gerookte sigaretten tijdens de zwangerschap, geassocieerd met gunstige effecten op het geboortegewicht.

Omdat een laag geboortegewicht slechts een afgeleide is van foetale groeivertraging, is het niet de beste maat om de nadelige effecten van roken tijdens de zwangerschap op de foetale groei te onderzoeken. Foetale groeivertraging kan leiden tot een normaal geboortegewicht bij een foetus die, gezien het genetische groei potentieel, eigenlijk volgens de bovenste percentielen had moeten groeien. In **hoofdstuk 4.2** toonden we aan dat actief blijven roken van moeder tijdens de zwangerschap geassocieerd was met een verminderde groei van de foetale hoofdomtrek, buikomtrek en femurlengte. Deze verminderde foetale groei leidde tot een kleinere femurlengte vanaf halverwege de zwangerschap en een kleinere hoofdomtrek en buikomtrek vanaf laat in de zwangerschap. De eerder optredende en grotere effecten op de femurlengte suggereren dat roken van moeder tijdens de zwangerschap primair de perifere weefsels beïnvloed. Roken van moeder totdat de zwangerschap bekend was, had geen nadelige invloed op de foetale groeipatronen.

Overmatig alcoholgebruik tijdens de zwangerschap is geassocieerd met verschillende zwangerschapscomplicaties zoals laag geboortegewicht, vroeggeboorte en congenitale afwijkingen. De effecten van overmatig alcoholgebruik tijdens de zwangerschap kunnen niet makkelijk geëxtrapoleerd worden naar het effect van gebruik van alcoholische dranken in mindere mate. Onze bevindingen, gepresenteerd in **hoofdstuk 4.3**, lieten zien dat alcoholgebruik van moeder van minder dan één consumptie per dag niet geassocieerd was met een toegenomen risico op een laag geboortegewicht en vroeggeboorte. Voor alcoholgebruik van één of drie drankjes per dag werd een tendens naar een toegenomen risico op laag geboortegewicht en vroeggeboorte gevonden.

Onderzoek in **hoofdstuk 5** bestudeert de effecten van foetale groeivertraging en roken van moeder tijdens de zwangerschap op de postnatale ontwikkeling van risicofactoren voor hart en vaatziekten.

Linker ventrikelhypertrofie is een sterke en onafhankelijke risicofactor van morbiditeit en mortaliteit. Aangezien het hart zijn grootste groeisnelheid heeft in de foetale en vroeg postnatale fase, zou een ongunstige omgeving in deze periode de groei en ontwikkeling van de linker ventrikel kunnen beïnvloeden. Het onderzoek gepresenteerd in **hoofdstuk 5.1** werd uitgevoerd in een subgroep van het Generation R cohort en liet positieve associaties zien van de buikomtrek laat in de zwangerschap en het geboortegewicht met de linker ventrikelmassa en de diameter van de aortabasis op de vroege zuigelingenleeftijd. Deze associaties waren onafhankelijk van het huidige gewicht en lengte en suggereren dat een kleinere foetusgrootte laat in de zwangerschap geassocieerd is met een persisterende kleinere linker ventrikelmassa en diameter van de aorta-

basis op de vroege zuigelingenleeftijd. Verder onderzoek is nodig om na te gaan of en in welke mate deze cardiale veranderingen persisteren op latere leeftijd.

Lange termijn follow-up onderzoek werd uitgevoerd in het Epidemiologisch Preventief Onderzoek Zoetermeer (EPOZ), een populatiegebaseerd prospectief cohort onderzoek in 596 kinderen, oorspronkelijk in de leeftijd van 5 tot 19 jaar. De huidige follow-up in dit onderzoek is 27 jaar.

Eerder onderzoek suggereerde dat roken van moeder in de zwangerschap geassocieerd is met een toegenomen bloeddruk bij de kinderen. Dit onderzoek werd verricht bij kinderen tot de leeftijd van 9 jaar. Het onderzoek gepresenteerd in **hoofdstuk 5.2** liet zien dat roken van moeder in de zwangerschap geassocieerd was met toegenomen jaarlijkse veranderingen in systolische en diastolische bloeddruk bij de kinderen. Deze toename leidde tot een hogere systolische en diastolische bloeddruk vanaf de jong volwassenheid. De associaties werden niet verklaard door een laag geboortegewicht. Dit suggereert dat een andere onderliggend mechanisme van invloed is.

In hetzelfde onderzoek onderzochten we de associaties van roken van moeder tijdens de zwangerschap met de ontwikkeling van het totale cholesterol, HDL-cholesterol en LDL-cholesterol vanaf de kinderjaren tot in de volwassenheid. In **hoofdstuk 5.3**, toonden we voor het eerst aan dat roken van moeder tijdens de zwangerschap geassocieerd was met een toegenomen jaarlijkse stijging van het totaal cholesterol vanaf de kinderjaren tot in de volwassenheid en een neiging naar een ongunstig lipoproteine profiel bij het nageslacht. Het effect van roken van moeder tijdens de zwangerschap op de totale cholesterol ontwikkeling was beperkt tot kinderen in de hoogste body mass index groep.

Toegenomen arteriële vaatwandstijfheid draagt bij aan de ontwikkeling van hypertensie en zou één van de mechanismen kunnen zijn die de associatie tussen karakteristieken in het vroege leven en bloeddruk op latere leeftijd verklaren. Eerder is de hypothese geformuleerd dat een ongunstige foetale omgeving leidt tot zowel groeivertraging als afgenomen elastine synthese in de grote arteriën en vervolgens tot permanente stijvere arteriën en een toegenomen bloeddruk later in het leven. Deze hypothese werd getest in het onderzoek gepresenteerd in **hoofdstuk 5.4**. Resultaten van dit onderzoek suggereren dat het causale verband voor de eerder gevonden associaties van roken van moeder tijdens de zwangerschap, laag geboortegewicht en vroeggeboorte met een toegenomen bloeddruk op latere leeftijd in het leven andere mechanismen omvat dan arteriële stijfheid.

In **hoofdstuk 6**, de algemene discussie, worden de resultaten van dit proefschrift beschreven en in een bredere context geplaatst. Bovendien worden relevante methodologische kwesties bediscussieerd en suggesties gegeven voor toekomstig onderzoek.

Dankwoord

Onderzoek zoals in dit proefschrift beschreven, kan alleen maar uitgevoerd worden dankzij de deelname van duizenden ouders en kinderen, de uitvoering door vele medewerkers en de creatieve ideeën van betrokken wetenschappers. Daarom is het eerste woord van dank aan deze grote groep personen gericht. Helaas te veel om persoonlijk te noemen. Samen hebben zij ervoor gezorgd dat Generation R zich heeft ontwikkeld van een spannend avontuur tot een goudmijn voor toekomstig onderzoek.

Jacqueline Witteman, dank voor de ruimte die je me gaf en het vertrouwen dat je altijd maar weer in me had. Ook op momenten dat ik dat zelf nogal onterecht vond. De afgelopen jaren heb ik zeer veel van je geleerd. Mooi dat het gelukt is een echte fetal-origins onderzoekslijn op te zetten. Bert Hofman, ik realiseer me dat het een gedurfde keuze was om veel verantwoordelijkheden te geven aan een onderzoeker die zelf nog moet promoveren en een klinische opleiding doet. Dank voor het vertrouwen. Jacqueline en Bert, door jullie heb ik altijd het heerlijke gevoel gehad in het land van de onbegrensde mogelijkheden te werken. Met jullie combinatie heb ik de beste opleiding in de populatie-epidemiologie gehad die ik me kon wensen.

Hans Büller, weliswaar de laatste tijd van iets grotere afstand, dank voor je betrokkenheid bij mijn eigen project en je altijd aanwezige bereidheid tijd vrij te maken voor Generation R wanneer er weer eens een beroep op je wordt gedaan. Eric Steegers en Bert van der Heijden, dank voor het beoordelen van het manuscript.

David Barker, I am most grateful for the opportunity you gave me to work at your MRC unit. This was a very inspiring period for me. Thank you for reviewing the manuscript. Clive Osmond, your '10 principles of statistics I wished I learned while I was a student' are still very useful!

Maria de Ridder, dank voor de hulp bij de moeilijkere analysen. Lenny van Osch, je hebt veel geduld op moeten brengen, maar de leuke papers zitten er absoluut aan te komen! Annette van den Elzen, het was een verademing om met jouw schone dataset te werken. Dank voor de hulp bij het werken met de EPOZ data. Rosalinde Snijders, ik heb veel bewondering voor de wijze waarop je Generation R in een moeilijke fase van de grond hebt gekregen (Veel succes in Portugal?). Bert van der Heijden en Matthijs de Hoog, dank voor de bereidheid mee te werken om een combinatie van Generation R en de kindergeneeskunde voor mij mogelijk te maken.

Het Generation R team, de eerste promoties zijn vooral een kroon op jullie werk en, nog belangrijker, een startsein voor vele volgende promotiefeesten! Mattijs, knap hoe je het afgelopen jaar op je eigen manier invulling hebt gegeven aan jouw moeilijke functie. Koos, de -niet altijd zo- stille kracht, niet meer valsspelen bij gezellig bedoelde spelletjes voor de hele afdeling! De huidige vaste medewerkers van het bureau, Christy, Elianne, Elise, Gulvüz, Janneke, Jeanine, Jos, Katja, Laura, Lies, Margot, Marijke, Miranda, Patty,

Rineke, Rukiye, Rose, Souad en Tonie, dank voor jullie onmisbare inzet. De datamanager, Claudia en natuurlijk de datamanagers i.o. Hanan en Lindsay, jullie zorgen ervoor dat promoveren bij Generation R binnenkort, in ieder geval wat betreft de data, een stuk makkelijker wordt. De ICT-wizards, Edward, Herman, Pascual en Robert-Jan, gelukkig hangen jullie niet meer op het imaginaire dartbord. GeneratoR ziet er prachtig uit! Ben benieuwd naar de uitbreidingen. Eric en Nano, dank voor de hulp van een afdeling met iets meer ervaring en de computerondersteuning. Rachel, begonnen als datamanager zonder data en een nuchtere steun in een hectische fase, ik kijk uit naar onze volgende samenwerking na jouw jaar in Boston. De collega-promovendi, Anne, Annemarie, Anushka, Ashna, Bero, Carmelo, Ernst-Jan, Hanan, Jens, Jitske, Joost, Lamise Liesbeth, Lindsay, Marianne, Mijke, Miranda, Noortje, Pauline, Sabine en Tamara, na het invoeren van tienduizenden vragenlijsten, het borrelen op vrijdag, het bellen van duizenden deelnemers, het borrelen op donderdag, het tellen en ordenen van duizenden CB-formulieren, het borrelen op woensdag en al het logistieke werk en geborrel dat daar nog eens bij kwam, is het eindelijk tijd om echt onderzoek te doen (en te borrelen). Mijke, ik zal onze samen doorgebrachte weekenden, avonden en nachten op het net missen.

Ernst-Jan, Liesbeth en Rachel, mijn alternatieve kleine-commissie, dank voor het lezen van het manuscript. Heel handig een tijdsverschil in de laatste nachten voor een deadline!

Mijn paranimfen. Ernst-Jan, dank voor je hulp in de laatste fase en bovenal een mooie vriendschap. Zonder jouw Coenen-Crisis overleg, harde deadlines en inzet voor de promotie, was ik waarschijnlijk nu nog bezig met heranalyseren en hadden we het tijdens de promotie moeten doen met een op het laatste moment uitgeprinte stapel stencils. Joep, jij richtte je meer op de inhoud. Van bijna iedereen die het manuscript las, stelde jij de leukste vragen. Eindelijk heb je besloten te gaan promoveren en je nog verder te gaan ontwikkelen in een, zoals je zelf aangeeft, zeer dynamisch vakgebied.

Mijn Leidse (inmiddels ook Alphense (?!) en Rotterdamse) vrienden. Al meer dan 10 jaar bij elkaar. Het is even wennen dat de vakanties met steile pistes, obscure disco's in de Alpen, harde rukwinden en woeste wateren zijn ingeruild voor het rustige genieten in een weekendhuisje terwijl we de opgroeiende kinderen evalueren. Ik kijk alweer uit naar het volgende weekend of weekje. In principe kan ik mee. Staat 24 mei al op het borrelrooster?

Pa, Grace, Raymond en Gillian. Bedankt voor de kansen, de altijd aanwezige gezelligheid en warmte en vooral het lekkere eten. Heerlijk om altijd de jongste te blijven.

Liesbeth, elke toelichting zou je tekort doen. Bedankt voor alles.

About the author

Vincent Jaddoe was born on October 11, 1974 in Zaandam, The Netherlands. He passed his secondary school in 1993 at the Zaanlands Lyceum (gymnasium) in Zaandam. In the same year, he started to study medicine at the Leiden University Medical Center (LUMC). After obtaining his medical degree in 1999, he started the work described in this thesis at the Department of Epidemiology & Biostatistics, Erasmus Medical Center, Rotterdam (head Prof.dr. A. Hofman). During this period, he worked 5 months as research-fellow at the Medical Research Council, Environmental Epidemiology Unit at the University of Southampton (head Prof. D.J.P. Barker) and he worked 1 year as a resident in pediatrics at the Sint Franciscus Hospital, Rotterdam (head Dr. R. Spritzer). From April 2003, he combined his research training with a residency in pediatrics at the Erasmus Medical Center, Sophia Children's Hospital (head pediatrics Prof.dr. A.J. van der Heijden, Dr. M. de Hoog). In 2005, he obtained a Master of Science degree in Clinical Epidemiology from the Netherlands Institute for Health Sciences (Nihes) in Rotterdam. He received the Nihes Award for the best scientific article during the academic year 2004 – 2005. Since August 2005, he is appointed as coordinator of the Generation R Study.