Preterm birth, early growth and adult metabolic health

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1 General introduction

Background

Small-for-gestational-age (SGA) is mostly the result of intrauterine growth retardation (IUGR), a process of slow fetal growth velocity according to intrauterine growth diagrams used in obstetrics (1). Different cut-off levels for SGA can be found in the literature, including birth size below the 10th or 5th percentile. By international consensus in 2001, SGA is defined as a birth weight and/or length of >2 SDs below the sex-specific population reference mean for gestational age (2). This was confirmed by a recent consensus meeting (3).

Being born SGA is associated with a short final stature (<-2 SD score, SDS), especially among persons who failed to catch up in height before the age of 2 years. However, at least 85% of SGA children have a height >-2 SDS at age 2 years (4;5). Those who fail to catch up within 2 years after birth have a 7- to 10-fold increased risk of becoming short as adult (4;5). Growth hormone (GH) treatment has in recent years become available for short children born SGA.

Poor intrauterine growth is not only associated with a short stature. A number of studies, initiated by Prof. David Barker, have demonstrated associations between lower birth weight and cardiovascular mortality, non-fatal cardiovascular diseases, and narrowing of the carotid artery (6-8). Lower birth weight has also been associated with several risk factors for cardiovascular disease, such as insulin resistance, dyslipidaemia, and hypertension (reviewed in (9-12)). Furthermore, it has been shown that persons with accelerated weight gain in infancy after a lower birth weight for gestational age were the most insulin-resistant (13;14).

Several hypotheses have been proposed as explanations for these associations. The "thrifty phenotype" hypothesis, proposed by Barker's group, postulates that the undernourished fetus responds with (permanent) ß-cell hypoplasia and peripheral insulin resistance in order to increase central nutrient availability at the expense of somatic growth (15). The "fetal salvage" hypothesis offers a similar explanation but is dissimilar regarding the ß-cell hypoplasia, which is, according to this hypothesis, not a part of the adaptive response of the fetus to intrauterine malnutrition (16). The "catch-up growth" hypothesis postulates that the insulin resistance after IUGR develops in neonatal life to protect the small newborn against hypoglycaemia, when abundant food supply leads to markedly elevated concentrations of insulin and insulin-like growth factor-I (17). Because fetal growth is in part insulin-mediated, the "fetal insulin" hypothesis postulates that lower birth weight is an epiphenomenon of type 2 diabetes susceptibility genes (18-20). Increased glucocorticoid bioactivity has also been proposed as an explanation for the associations, since there is a clustering of Cushingoid features after lower birth weight (21).

Rationale for this thesis

In The Netherlands, like in most industrialized countries, there is a rising incidence in the number of preterm births. This is attributed to an older maternal age at first birth, more widespread application of assisted reproduction technologies, and, hence, more twin gestations. Nowadays, 1% of children is born at a gestational age <32 weeks (22). Furthermore, local data indicate that, in comparing the years 1983 and 1996-1997, neonatal survival has increased from 70% to 89% for infants born <32 gestational weeks (23). The implication of these changes is that the number of preterm infants reaching adulthood will rise in the following years.

There is some evidence for analogies in the endocrine-metabolic state of preterm infants and children born SGA. At 7 years of age, prematurely born children were, regardless of their intrauterine growth, found to be as equally insulin-resistant as term children born SGA (24). However, another study in prematurely born children aged 6 years found that those with birth weights below the 10th percentile were the most insulin-resistant (25). Nonetheless, follow-up of preterm populations into adulthood has not been performed yet. Therefore, it remains uncertain whether the observed associations persist into adult life.

The work presented in this thesis explores in individuals born very preterm (i.e., <32 gestational weeks) the effect of early growth on subsequent height development and the adult metabolic profile, as well as it investigates a few candidate pathophysiological mechanisms for the observed associations.

Population

The studies described in this thesis were conducted in the Project On Preterm and Small-for-gestational-age infants (POPS) cohort, which was recruited in 1983 and comprised of 94% of the very preterm (<32 gestational weeks) and/or very-low-birth-weight (<1,500 g) infants born in The Netherlands in that year (26). The main objective of the POPS study was to assess general and disease-specific mortality of such infants. From birth onwards, follow-up was continued which enabled to study handicaps, cognitive performance, linear growth, and various other characteristics (27). In 1999, an initiative was launched to study the POPS cohort at young adult age. Assessments took place between April 2002 and May 2003, at 19 years of age. Among others, venous blood was obtained after an overnight fast, anthropometry was performed, and blood pressure and carotid intima-media thickness (CIMT) were measured. The response rate was 62% (28).

Oultine of this thesis

Chapter 2 discusses the rationale for GH treatment in short children with a history of neonatal growth retardation after preterm birth. Chapter 3 compares the growth pattern up into adulthood between preterm infants born SGA and those with an appropriate birth size for gestational age followed by neonatal growth retardation. Chapters 4 to 7 address the effects of early growth on adult body composition (chapter 4), CIMT (chapter 5), the serum lipid profile (chapter 5), insulin resistance (chapter 6), and blood pressure (chapter 7). Chapter 8 reports on the effect of maternal glucocorticoid treatment, given for impending preterm delivery, on the adult metabolic profile of her offspring. Chapter 9 addresses the effects of 2 glucocorticoid receptor polymorphisms on the growth pattern and the adult metabolic profile. Chapter 10 presents a meta-analysis of published reports on the association between birth weight and basal cortisol level. Chapter 11 gives a brief overview of the major findings and limitations of the work presented in this thesis, and discusses the implications of these findings for patient care.

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2 Preterm-growth-restraint:

a paradigm that unifies intrauterine growth retardation and preterm extrauterine growth retardation and has implications for the small-for-gestational-age indication in growth hormone therapy

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Abstract

In contrast to children born small-for-gestational-age (SGA), preterm infants with normal size at birth who experienced neonatal growth retardation as part of a stormy postnatal course, resulting in a small size at term, are excluded from growth hormone (GH) therapy if they fail to catch up in height subsequently. Here, we question whether the time has come to update the SGA indication for GH therapy, which requires a birth weight or length >2 SDs below the mean for gestational age, into a preterm-growth-restraint indication, so that this group is no longer excluded from GH therapy in case of persistent short stature.

Small-for-gestational-age (SGA) is defined as a birth weight and/or length >2 SDs below the sex-specific population reference mean for gestational age. However, there is confusion about various aspects of this term, as recently discussed (1;2). The term "intrauterine growth retardation" (IUGR) is often used for the same condition but preferably should be restricted to poor growth during pregnancy according to intrauterine growth diagrams used in obstetrics (3). SGA after a normal duration of gestation (37 to 42 weeks) is usually followed by rapid growth after birth (catch-up growth). It has been demonstrated that almost 90% of term SGA infants catch up in height in the first 2 years of postnatal life (4;5).

On average, the human male has a birth length of 51 cm after term gestation, and a final height, in The Netherlands, of 184 cm. Thus, in the 9 months before birth, he has reached almost 30% of his adult height potential. Fetal length velocity at mid-gestation is >10-fold higher than pubertal peak height velocity (Figure 1).

Thus, very preterm infants are exposed to extrauterine life during a period that is normally characterized by rapid intrauterine growth. To survive, their energy expenditure shifts from growth-promoting actions to survival strategies to cope with the increased requirements of unintended postnatal life. Extrauterine growth retardation (EUGR) is often the result. Preterm infants whose mothers suffered from conditions such as preeclampsia are usually already growth-retarded at birth. Nonetheless, regardless of whether the child is born SGA, very preterm infants tend to be small at term, and a considerable proportion of them even meet criteria for SGA by that age. A study among 52 children born before 29 weeks' gestation showed that 13 (25%) had length at term <-2 SDs (6).

Figure 1. Normal length/height velocity from conception to adulthood (boys).

Fetal length velocity reaches its maximum during mid-gestation, 10 cm/month, and declines to 35 cm/year around birth. In comparison, the median for peak height velocity during puberty is 9.42 cm/year. Postnatal height velocity (median values) is according to Dutch reference values (13).

Thus, among non-syndromatic children with growth retardation before term age, 3 major groups can be differentiated: (I) term children born SGA as a result of IUGR, (II) children born (very) preterm with appropriate size for gestational age who experienced EUGR as part of a stormy neonatal course, and (III) children born (very) preterm who experienced IUGR resulting in being SGA and experiencing EUGR.

According to current legislation across Europe, the second of these groups is excluded from growth hormone (GH) therapy in case of persistent short stature, because these children were excluded – for unspecified reasons – from the pivotal studies that were initiated around 1990, and were maintained up to adult height (7). Here, we question whether the time has come to update the SGA indication for GH therapy, which requires a birth weight or length <-2 SDs for gestational age, into a preterm-growth-restraint (PGR) indication, so that this group is no longer excluded. Approximately 10% of very preterm children have a height <-2 SDs at 4 to 5 years of age (6;8). This is similar to the number of term SGA infants who do not show postnatal catch-up growth (4).

Because neonatal intensive care is a relatively recent and rapidly evolving discipline, there was until now a virtual "absence of evidence" for analogies among the 3 aforementioned groups. Thanks to a set of recent data, this absence of evidence is gradually changing into an "evidence of absence" of major differences between the endocrine-metabolic state of the second group versus that of the other 2 groups. To date, this evidence already includes key features, such as body composition (9;10), insulin sensitivity (11), and blood pressure (12). Beyond the age of approximately 6 to 8 years, the children in these 3 groups seem to resemble each other so closely that, in the absence of a perinatal history, they are virtually indistinguishable from each other on clinical, biochemical, endocrine, and metabolic grounds.

Given that the short-term growth response to exogenous GH in this context may not be indicative of the long-term response (7), there are now 2 major ways to explore GH therapy in former premature infants with short stature. The "absence of evidence for a parallellism" option implies the initiation of long-term studies up to adult height (outcome known around the year 2020). The "evidence of absence of a difference" option would imply an extension of the SGA to a PGR indication, provided the results are monitored until such extension is conclusively validated.

We suggest that paediatric societies, including the American Academy of Pediatrics, the American Pediatric Society/Society for Pediatric Research, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology issue a statement on this specific topic. Below are a few elements to consider in the anticipated debate.

Etiology of PGR – The cause of an intrauterine growth failure that leads to the SGA condition of a baby born at or near term remains often unknown. As a rule, however, the common final pathway includes acidosis, hypoxia, and the equivalent of a fasting state, with serum levels that are low for insulin, insulin-like growth factor-I, and insulin-like growth factor-binding

protein-3 and high for insulin-like growth factor-binding protein-1 and GH. Extrauterine growth failure of very-low-birth-weight premature infants is often attributed to some combination of factors, including low caloric intake, infections, respiratory distress, and pharmacological effects (e.g., of glucocorticoids).

Timing of PGR – In SGA infants born at or near term, it is often unknown whether the PGR started in the last trimester or earlier. In preterm neonates, direct documentation of the extrauterine growth failure is possible (weight, length, and head circumference). In other words, in term SGA infants, the PGR may or may not have started before the third trimester, whereas in preterm appropriate-for-gestational-age (AGA) newborns, the PGR is usually known to have occurred during the third trimester.

Etiology and timing of PGR: relevance for the SGA indication – In the current SGA indication for GH therapy, little attention is given to the etiology of the SGA condition (syndromatic conditions, however, are excluded) or to the timing (early versus late gestation) of the PGR. Given that the net impact of an early growth-restraining insult on subsequent stature may depend more on its timing than on the nature of the insult, the only change that we propose in the current SGA indication for GH therapy is that the timing of the growth restraint should be judged at term age rather than at birth.

Terminology and implementation – We propose to maintain the well-established indicators of size at birth, such as SGA, AGA, and large-for-gestational-age (LGA), and to complement them – for preterm infants – with their equivalents at term: small-at-term (SAT), appropriate-at-term (AAT), and large-at-term (LAT). According to this terminology, the proposed change in the SGA indication for GH would result in a SAT indication for GH therapy. Implementation of this approach would require that neonatologists include "size at term" as an obligatory part of their follow-up (weight, length, and head circumference), and always include information on this size at term in their reports of (very) preterm infants. Such data could then be used as indices of growth in the first 40 weeks if ever the option of GH therapy has to be contemplated because of persistent short stature in childhood.

Impact of a switch from an SGA to a PGR or SAT indication for GH therapy – On the basis of our clinical experience, we estimate that the quantitative impact of an extension from an SGA to a PGR indication will be in the range of 10%. In fact, thus far, the limited number of preterm AGA children with persistent short stature (8) has restrained academic centers, including ours, and the pharmaceutical industry from engaging in long-term GH studies within this patient population.

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3 Long-term height gain of prematurely born children with neonatal growth restraint:

parallellism with the growth pattern of children born small-for-gestational-age

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Abstract

Objectives:

It is unknown whether children born very preterm (<32 weeks' gestation) with appropriate size for gestational age (AGA), who grow poorly in the first postnatal months (i.e., preterm-growth-restraint, PGR), show a similar growth pattern as children born small-for-gestational age (SGA). In this study, childhood growth and adult height of very preterm AGA PGR children were compared to those of very preterm SGA and AGA non-PGR children.

Methods:

Data were drawn from the Project On Preterm and Small-for-gestational-age infants cohort. PGR was considered to have occurred after AGA birth, if length and/or weight was <-2 SD score (SDS) at the age of 3 months post-term.

Results:

Among 380 very preterm children, 274 experienced no PGR and showed near-normal growth, whereas 79 (21%) experienced PGR and subsequently displayed a growth pattern similar to that of very preterm SGA children (N=27). Adult height of these children was -1.1 to -1.2 SDS. Very preterm AGA PGR and SGA children with a height <-2 SDS at 5 years had an adult height of approximately -2.5 SDS.

Conclusions:

Childhood growth and adult height were similar in very preterm SGA and AGA PGR children. These long-term findings further strengthen the plausibility of extending the SGA indication for growth hormone (GH) therapy in such a way that very preterm AGA PGR children are no longer excluded from GH therapy, if they have a short stature persisting beyond the age of 5 years.

Introduction

There is increasing evidence that children who experienced a transient phase of preterm-growth-restraint (PGR) display a persistent ensemble of sequelae that are independent of whether the PGR occurred in utero (resulting in a small-for-gestational-age (SGA) infant), ex utero (preterm birth followed by poor neonatal growth), or in both perinatal phases. To date, this evidence already encompasses features like body composition, insulin sensitivity, and blood pressure (reviewed in Wit et al (1)). From the age of approximately 6 to 8 years onward, the PGR subgroups converge their pathophysiological patterns so that, in the absence of a perinatal history, they become nearly indistinguishable on clinical, biochemical, endocrine, or metabolic grounds. Here, we extend this concept further: up to adult height, the growth pattern of very preterm appropriate-for-gestational-age (AGA) PGR children was compared with that of very preterm SGA and AGA non-PGR children.

Methods

Population

Growth data were extracted from the prospective Project On Preterm and Small-for-gestational-age infants (POPS), which follows a nationwide cohort of very preterm (<32 weeks' gestation) and/or very-low-birth-weight (<1,500 g) infants born in The Netherlands in 1983 (2). For this specific study, only the very preterm non-syndromic white subjects were included, provided that their growth data (length and weight) at birth and at 3 months post-term were complete (N=380). Size at 3 months post-term was used as proxy for size at term.

Study protocol

Length until the age of 2 years post-term was measured to the nearest 1 cm in supine position, fully extended with the heels in contact with a baseboard. At ages 5 and 19 years, standing height was measured to 1-mm accuracy. Sizes at birth and beyond birth were converted to SD score (SDS) using Swedish and Dutch references, respectively (3;4).

By definition (5), very preterm subjects (<32 gestational weeks) with birth length and/or weight <-2 SDS were classified as SGA, whereas those with birth length and weight ≥-2 SDS and length and/or weight at 3 months <-2 SDS were labelled as AGA PGR, and those with birth length and weight ≥-2 SDS and length and weight at 3 months also ≥-2 SDS were labelled AGA non-PGR. Target height was calculated as: mid-parental height + 6.5 (-6.5 for females) + 4.5 cm (correction for Dutch secular trend). Ethical approval and written informed consent was obtained from all of the participants.

Statistical analysis

All of the auxological data were normally distributed. Length/height measurements were compared between AGA PGR and AGA non-PGR groups, as well as between AGA PGR and SGA groups, using the unpaired t test. These comparisons were repeated after adjustment for perinatal morbidity, using linear regression analysis. Statistical significance was defined as a P value <0.05. To adjust for possible bias caused by the relatively greater availability of growth data of taller persons at the age of 19 years, missing data for adult height SDS was predicted from the available height SDS data at 5 years through imputation for each group separately by linear regression analysis.

Results

There were 1,338 children in the original cohort, of whom 1,012 were born before 32 weeks of gestation. Of those, 683 were still alive at the age of 1 year. After exclusion of 7 syndromic children and persons from non-white ancestry, 571 subjects were left. Complete data for size (length and weight) at birth and at 3 months post-term was available for 380 children. The

Table 1. Prevalence of prenatal and postnatal characteristics of very preterm infants.

Characteristic	SGA	AGA PGR	AGA non-PGR	P	
				AGA PGR vs. AGA non-PGR	AGA PGR vs. SGA
N	27	79	274		
Obstetric					
Parity > 0 (%)	10 (37%)	44 (56%)	137 (50%)	0.32	0.08
Multiple pregnancy (%)	3 (11%)	28 (35%)	65 (24%)	0.04	0.02
Maternal hypertension (%)	12 (44%)	15 (19%)	36 (13%)	0.19	0.009
Gestational diabetes (%)	0	5 (6%)	18 (7%)	0.96	0.33
Maternal smoking (%)	12 (44%)	23 (32%)	99 (38%)	0.34	0.25
Maternal intake of drugs/alcohol (%) [a]	18 (67%)	45 (57%)	157 (57%)	0.96	0.38
Neonatal					
Gestational age (weeks)	30.9 (28.3 to 31.9)	29.3 (25.4 to 31.7)	30.4 (25.6 to 31.9)	<0.001	<0.001
Respiratory distress syndrome (%)	11 (41%)	55 (70%)	134 (49%)	0.001	0.008
>7 Days on assisted ventilation (%)	5 (19%)	37 (47%)	50 (18%)	0.02	0.009
Intracranial hemorrhage (%)	5 (19%)	22 (28%)	36 (13%)	0.002	0.34
Convulsions (%)	0	5 (6%)	8 (3%)	0.16	0.18
Postnatal corticosteroids (%)	2 (7%)	11 (14%)	10 (4%)	0.002	0.51
Sepsis (%)	10 (37%)	26 (33%)	74 (27%)	0.31	0.70
Necrotising enterocolitis (%)	6 (22%)	4 (5%)	9 (3%)	0.50	0.02

Values represent N (%) or median (range). Continuous variables were compared with the unpaired t test.

Dichotomous variables were compared by the χ^2 test or Fisher's exact test.

[a] Smoking, drinking alcohol, or using soft drugs, hard drugs or methadone during pregnancy.

AGA PGR condition (N=79; 21%) was 3-fold more prevalent than SGA (N=27; 7%). Among AGA PGR children, 22 were PGR for weight, 21 for length, and 36 for both.

Table 1 lists a selection of conditions that may have contributed to prenatal and/or postnatal growth restraint. Comparing AGA PGR with AGA non-PGR and SGA children, the AGA PGR group was characterized by a low gestational age and, accordingly, by a high prevalence of respiratory distress syndrome and prolonged ventilation. There was also a greater proportion with intracranial hemorrhage and on glucocorticoid therapy among AGA PGR children than among those born AGA without PGR.

Table 2 summarizes the growth patterns of the groups up to adult height. The growth patterns of AGA PGR and SGA groups were similar from the age of 3 months post-term onwards. At birth, AGA PGR children were somewhat shorter and lighter than AGA non-PGR children. Throughout childhood, stature of AGA PGR children was shorter than that of AGA non-PGR children. These differences persisted after correction for the variables listed in Table 1 (data not shown).

Table 2. Spontaneous growth of very preterm infants.

Variable	SGA			AGA PGR			AGA non-PGR			P	
	N	Median	Range	N	Median	Range	N	Median	Range	AGA PGR vs. AGA non-PGR	AGA PGR vs. SGA
Neonatal size SDS											
Birth weight	27	-2.1	-3.2 to 0.7	79	-0.2	-2.0 to 1.3	274	0.1	-2.0 to 2.7	<0.001	<0.001
Birth length	27	-2.5	-4.4 to -1.4	79	-0.2	-1.6 to 2.8	274	0.2	-2.0 to 3.6	0.01	<0.001
3 mo eight	27	-2.6	-5.3 to 0.4	79	-2.3	-4.2 to 0.3	274	-0.3	-2.0 to 2.6	<0.001	0.61
3 mo lenght	27	-2.4	-5.4 to 0.2	79	-2.3	-4.8 to -0.8	274	-0.4	-2.0 to 3.3	<0.001	0.31
Length/height SDS at follow-up visits											
1 yr	26	-1.6	-5.6 to 0.3	71	-1.3	-5.5 to 2.8	252	-0.3	-6.0 to 2.3	<0.001	0.18
2 yrs	26	-1.2	-4.8 to 1.2	70	-1.1	-4.5 to 1.4	244	-0.2	-2.8 to 4.3	<0.001	0.76
5 yrs	27	-1.0	-3.1 to 1.6	75	-0.7	-4.6 to 1.6	259	-0.1	-4.1 to 2.7	<0.001	0.40
19 yrs (available data)	19	-0.8	-2.9 to 0.7	51	-0.8	-2.7 to 0.4	157	-0.3	-2.9 to 2.1	<0.001	0.88
19 yrs (with data imputation)	27	-1.2	-2.9 to 0.7	76	-1.1	-3.9 to 0.4	264	-0.4	-3.4 to 2.1	<0.001	0.92
Target height SDS	27	-0.2	-1.6 to 1.0	77	-0.1	-1.6 to 1.5	270	0.1	-2.1 to 2.4	0.06	0.36

Table 3 shows that, among AGA PGR children, the prevalence of short stature is close to 20%, as it is among very preterm SGA children. A short stature at the age of 5 years in these 2 groups points to a high risk (~90%) of short stature in adulthood, whereas a stature ≥-2 SDS at 5 years old was associated with a low prevalence (~10%) of short stature in adulthood. AGA PGR and SGA children with a height <-2 SDS at the age of 5 years had a median adult height of approximately -2.5 SDS.

Discussion

In this population-based study of very preterm children, the AGA non-PGR children displayed a virtually normal growth pattern, whereas the AGA PGR and SGA children grew in a way that has previously been described for SGA children born at term (6;7). The present data are the first to document the spontaneous growth pattern of AGA PGR children up to adult stature. Hence, they are also the first to evidence that AGA PGR children, if still short (height <-2 SDS) at the age of 5 years, have a similar risk (~90%) to become short adults as do SGA children (whether born preterm or not) who are still short at that age. The striking long-term parallellism between AGA PGR children and SGA children is herewith extended to linear growth up into adulthood.

The present findings corroborate the rationale to extend the current growth hormone (GH) indication for short SGA children in such a way that it harbours also those very preterm born AGA PGR children who still have a height <-2 SDS at the age of 5 years. Departing from the numbers in this article, it can be estimated that a PGR extension of the current SGA indication for GH would increase the number of eligible children by 10 %. Because average adult height SDS is very close to mean height SDS in childhood and as younger children respond more to exogenous GH (8), such therapy should preferably start at an early age.

In our study, no bias could have been introduced by the high neonatal mortality rate (2), since the indication for GH therapy is determined beyond the toddler age range. However, because mortality of very preterm infants has dramatically declined between 1983 and the mid-1990s, especially because of a reduction in mortality from respiratory distress syndrome (9), the sicker children – presumably with PGR – survive nowadays. This increasing survival rate has also resulted in a rising incidence of bronchopulmonary dysplasia (9), which implies that the prevalence of short stature may be higher in the next generation of very prematurely born children.

Conclusions

In conclusion, prematurely born children who experienced PGR were found to have a growth pattern similar to that of SGA children. These data corroborate a concept in which short AGA PGR children are considered to be pathophysiological equivalents of short SGA children. The present evidence undermines the current policy to exclude PGR survivors from GH therapy if their small size evolves toward a short stature in childhood.

Table 3. Short stature at age 5 years points in AGA PGR and in SGA children to a high risk (~90%) of short stature in adulthood; conversely, a stature ≥-2 SD at age 5 years implies a low risk (~10%) of short stature in adulthood.

Height SDS at 5 yrs	SGA		AGA PGR	
	<-2 SDS	≥-2 SDS	<-2 SDS	≥-2 SDS
N	6	21	11	64
Height SDS at 19 yrs	-2.6 (-2.9 to -1.8)	-0.8 (-2.1 to 0.7)	-2.4 (-3.9 to -1.4)	-0.8 (-2.7 to 0.4)
N <-2 SDS at 19 yrs (%)	5 (83%)	1 (5%)	10 (91%)	7 (11%)

Values represent median (range) or N (%).

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4 Preterm birth and body composition in adulthood:

different effects of intrauterine and infancy weight gain

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Abstract

Objectives:

Increasing evidence indicates that adult body composition is associated with prenatal and infancy weight gain, but the relative importance of different time periods has not been elucidated. Therefore, we studied the association between prenatal, early postnatal, and late infancy weight gain, and BMI, fat mass, and fat distribution in young adulthood.

Methods:

We included 403 men and women aged 19 years from a Dutch national prospective follow-up study who were born at a gestational age <32 weeks. BMI SD score (SDS), waist circumference SDS, and waist-to-hip ratio SDS, and subscapular-to-triceps ratio, percentage body fat, fat mass, and fat-free mass at age 19 years were studied in relation to birth weight SDS, weight gain from preterm birth until 3 months post-term (early postnatal weight gain), and from 3 months until 1 year post-term (late infancy weight gain).

Results:

Birth weight SDS was positively associated with weight SDS, height SDS, BMI SDS, and fat-free mass at age 19 years but not with fat mass, percentage body fat, or fat distribution. Early postnatal and late infancy weight gain were positively associated with adult height SDS, weight SDS, BMI SDS, waist circumference SDS, fat mass, fat-free mass, and percentage body fat but not with waist-to-hip ratio SDS or subscapular-to-triceps ratio.

Conclusions:

In individuals born very preterm, weight gain before 32 weeks of gestation is positively associated with adult body size but not with body composition and fat distribution. More early postnatal and, to a lesser extent, late infancy weight gain are associated with higher BMI SDS and percentage body fat and more abdominal fat at age 19 years.

Introduction

Obesity is a major health problem throughout the world. Numerous studies have shown an association between obesity and various cardiovascular risk factors, such as diabetes, hypertension, and dyslipidemia (1-3). Obesity is also associated with an increased risk of death (4).

Fetal life and the early postnatal period have been suggested to be important for the development of adult obesity (5;6). The Dutch famine studies have shown that reduced maternal calorie intake during the first 2 trimesters of pregnancy might increase the risk of adult obesity (7;8). The association between birth weight, mainly an indicator of fetal growth during the third trimester, and adult obesity is equivocal (9). In several studies, a linear positive association has been found (10-12), whereas in others a J- or U-shape (13;14) or no association was observed (15). In these studies, obesity was expressed as BMI, which includes both fat mass and fat-free mass.

In studies about fat mass and fat distribution, low birth weight has been associated with a more central pattern of fat distribution (16;17) and a lower BMI, mostly because of a lower lean body mass and not a lower fat mass (18-22). In addition, a rapid rate of weight gain during early infancy has been associated with both a higher BMI (23) and more fatness and a more central pattern of fat distribution in childhood (6). In certain specific populations, early growth has been positively associated with obesity and lean body mass in adulthood (24;25). However, the associations between birth weight and adult body composition have not been consistently found in all populations (26;27), and in various studies the associations became significant only after adjustment for adult BMI (16;17;21;22). It is still unclear whether the associations found between early postnatal weight gain and fat mass and fat distribution in childhood persist into adulthood, and even less is known about fetal growth during the first 2 trimesters of pregnancy and subsequent adult body composition in humans.

We studied the relation between birth weight and early postnatal weight gain and adult BMI, fat mass, and fat distribution within the scope of the Project On Preterm and Small-for-gestational-age infants (POPS), a national cohort of individuals born very preterm. In this prospective study, birth weight could be used as an indicator of fetal growth during the first 2 trimesters, whereas growth during the third trimester and the period thereafter could be monitored well ex utero. We studied the relative predictive value of weight gain before 32 weeks of gestation, during the period from preterm birth until 3 months post-term (early postnatal weight gain), and from 3 months until 1 year post-term (late infancy weight gain) for BMI, fat distribution, and body composition at age 19 years.

Methods

Population

The subjects were participants of the POPS study. The POPS cohort comprises 94% of all live-born infants in The Netherlands between 1 January and 31 December 1983 after a gestation of <32 completed weeks, with a birth weight of <1,500 g, or both (28). The physical and psychosocial outcomes of the POPS cohort have been intensely studied over the years (28;29). In the current study, conducted when the subjects were 19 years of age, only those subjects with a gestational age <32 weeks were studied. Subjects with congenital malformations leading to changes in body proportions and body composition (e.g., focomely, amely, chromosomal abnormalities, and inborn errors of metabolism) were not eligible for inclusion. The study was approved by the medical ethics committee of all participating centers, and written informed consent was obtained from all participants.

Study protocol

Weight, length, and head circumference were measured at birth and expressed as SD score (SDS) to correct for gestational age and sex with the use of Swedish references for very preterm infants (30). At the ages of 3 months and 1 year post-term, weight and length were measured at the outpatient clinics of the participating centers by trained physicians and nurses. These measurements were expressed as SDS using Dutch reference values (31). Weight gain between birth and the age of 3 months post-term (early postnatal weight gain), and between the ages of 3 months and 1 year post-term (late infancy weight gain) were computed as delta-SDS.

Anthropometric measurements were performed in 10 centers in the Netherlands by 15 nurses and physicians according to standardized procedures when the subjects had reached the age of 19 years. All assessors had received extensive training before the start of the study; during the study, retraining and standardization were carried out at 2-months intervals to maximize interobserver reliability. Assessors were blinded with respect to the birth weight or duration of gestation of the subjects.

Subjects were measured barefoot while wearing underclothing. Weight was measured on a balance scale to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm with a fixed stadiometer. BMI was calculated as weight in kg/height^2 in cm (2). Waist circumference was measured at the level midway between the lower costal margin and the iliac crest after gentle expiration and hip circumference at the level of the greater trochanter, both with the use of a flexible tape measuring to 0.1-cm accuracy. The waist-to-hip ratio was calculated. Four skinfold thicknesses were taken in duplicate with a calibrated skinfold calliper on the left side of the body at the triceps, biceps, subscapular, and iliacal regions, according to guidelines of the World Health Organisation (biceps and subscapular) (32), and Falkner and Tanner (triceps and iliacal) (33). The sum of the 4 skinfold thicknesses was used as a measurement of overall

subcutaneous fatness. The ratio of subscapular-to-triceps-skinfold thickness was calculated as an index of truncal to peripheral adiposity (34). Fat mass and the corresponding fat-free mass were computed by using the equations of Durnin and Rahaman (35). All outcome measures at age 19 years, except for the derived outcomes, were expressed as SDS according to recent Dutch references (31;36;37).

Statistical analysis

Multivariate linear regression analyses were performed in SPSS 11.0 software (SPSS Inc, Chicago, USA) to assess associations between prenatal, early postnatal, and late infancy weight gain and the outcome measures at age 19 years. To disentangle the effects of birth weight, early postnatal weight gain, and late infancy weight gain on adult outcomes, early postnatal weight gain was corrected for birth weight, and late infancy weight gain was corrected for both the effect of birth weight and the effect of early postnatal weight gain. This correction was performed by entering the variables mentioned above into multivariate regression models. An interaction term, computed as the product of birth weight SDS and early postnatal weight gain and late infancy weight gain, respectively, was introduced to assess whether the effect of early postnatal and late infancy weight gain on outcome measures at age 19 years was different for those individuals with low birth weights compared to those with higher birth weights. The relative importance of weight gain during the various time periods was studied by comparing the changes in explained variance (R^2) for each period.

Because it was not possible to use an SDS for variables derived from skinfold thicknesses, regression analyses with these outcome measures were corrected for sex. The analyses with waist and hip circumferences, fat mass, and fat-free mass at age 19 years as outcomes were also adjusted for variations in adult body size by adjusting for current height SDS. The analyses with height SDS at age 19 years as outcome measure were adjusted for target height SDS, which was computed as: midparental height + 6.5 cm (-6.5 cm for females) + 4.5 cm (estimated secular trend per generation). All analyses were repeated with adjustment for the possible confounders race (white versus non-white), socio-economic status (measured on a 6-point scale in which 1 was lowest and 6 was highest), and physical activity (measured on a 3-point scale).

Results

In 1983, 1,012 infants who were born before 32 weeks of gestation were included in the POPS cohort; 669 without congenital malformations were still alive at age 19 years. Of these subjects, 415 (194 males and 221 females) gave informed consent for the present study (response rate 62%). No anthropometric measurements were performed in 8 subjects either because these subjects were wheelchair bound or because no calibrated instruments were available.

Four subjects were excluded from the analyses because of medical conditions or because they were taking medication that could lead to aberrations in body proportions and body composition: 2 subjects used oral corticosteroids, 1 woman had anorexia nervosa, and 1 woman was pregnant at the time of the study. The study population thus included 403 subjects in whom anthropometry was performed at age 19 years.

Characteristics of the subjects are given in Table 1. Non-response was higher among males, non-whites, and those with a mother with a low educational level. Birth weight SDS and gestational age did not differ significantly between responders and non-responders.

Table 1. Perinatal characteristics of participants and non-responders.

Characteristic	Participants	Non-responders
N	403	254
General		
Sex (% male)	46.4[b]	65.7
Race (% white)	87.7[c]	80.2
Low educational level mother (%)	38.9[b]	56.5
Obstetric		
Multiple birth (%)	22.8	21.7
Hypertension during pregnancy (%)	17.6	15.7
Diabetes mellitus gravidarum (%)	5.0	4.3
Smoking during pregnancy (%)	28.0	29.5
Drugs and intoxication (%)[a]	52.0	52.0
Elective delivery (%)	19.4[c]	13.4
Birth		
Gestational age (weeks)	29.7±1.5	29.8±1.5
Birth weight		
- g	1,316±336	1,347±274
- SDS	-0.13±1.0	-0.09±0.9
Birth length		
- cm	39.1±3.4[c]	39.6±2.9
- SDS	-0.12±1.2	-0.06±1.1
Head circumference at birth		
- cm	27.4±2.1	27.6±1.9
- SDS	0.03±1.2	-0.09±1.0
Postnatal		
Weight at 3 mo		
- kg	5.1±0.90	5.3±0.88
- SDS	-0.94±1.3	-0.90±1.4
Weight at 1 yr		
- kg	8.9±1.2	9.1±1.4
- SDS	-0.98±1.2	-0.94±1.4

Values represent mean±SD or percent. Continuous variables were compared with the unpaired t test.
Dichotomous variables were compared by the χ^2 test.
[a] Smoking, drinking alcohol, or using soft drugs, hard drugs or methadone during pregnancy.
[b] P <0.001 between participants and non-reponders.
[c] P <0.05 between participants and non-responders.

The anthropometric characteristics of the response group are provided in Table 2 as absolute values and SDSs. For both males and females, the mean values for height, weight, and BMI were lower than the means of the Dutch reference population of 19-year-olds, whereas the mean values for waist circumference, waist-to-hip ratio, and the sum of the skinfold thicknesses were greater than the Dutch population means.

The associations between prenatal, early postnatal, and late infancy weight gain and the anthropometric outcomes at age 19 years are shown in Table 3. Birth weight SDS was positively associated with adult height SDS, weight SDS, BMI SDS, and waist circumference SDS, although the 95% CIs for the latter 2 variables almost included 0. There was also a positive association between birth weight SDS and both fat mass and fat-free mass but not between birth weight SDS and percentage body fat at age 19 years. When adjusted for current height SDS, the association between birth weight SDS and waist circumference SDS disappeared. The regression coefficient of the association between birth weight SDS and fat-free mass decreased, and the association between birth weight SDS and fat mass became non-significant after correction for current height SDS. No significant associations were found between birth weight SDS and the waist-to-hip ratio SDS, the sum of 4 skinfold thicknesses SDS, and the subscapular-to-triceps ratio at age 19 years.

Early postnatal weight gain and late infancy weight gain were both positively associated with height SDS, weight SDS, BMI SDS, waist circumference SDS, fat mass, fat-free mass, and percentage body fat at age 19 years. Late infancy weight gain was also positively associated with the adult sum of 4 skinfold thicknesses SDS. The coefficients of waist circumference SDS, fat mass, and fat-free mass in relation to early postnatal and late infancy weight gain diminished after correction for current height SDS but remained significant. When adjusted for target height SDS, the associations between prenatal, early postnatal, and late infancy weight gain and adult height SDS remained significant but decreased in magnitude. No significant associations were found between early postnatal and late infancy weight gain and the waist-to-hip ratio SDS or subscapular-to-triceps ratio in young adulthood.

No significant interaction was found between birth weight SDS and early postnatal weight gain or between birth weight SDS and late infancy weight gain with regard to any of the outcome measures at age 19 years. Correction for race, socio-economic status, sex, and physical activity did not significantly change the results of the aforementioned analyses (data not shown).

For the anthropometric outcomes at age 19 years that were associated with weight gain during early life, the percentages of variance explained by weight gain during the different time periods are presented in Table 4. For current height SDS, 37.5% of variance was explained by target height SDS. Birth weight SDS explained 6.2% of the variance in current height SDS not explained by target height SDS, whereas early postnatal weight gain explained another 4.5% of current height SDS variance not explained by target height SDS or birth weight SDS. Late infancy weight gain explained 3.3% of the variance of current height SDS not explained

by the abovementioned variables. So, for current height SDS adjusted for target height SDS, the largest change in R^2 values was observed for the effect of birth weight SDS.

For adult weight SDS, the effect of birth weight SDS on R^2 change equalled the effect of early postnatal weight gain. For BMI SDS, waist circumference SDS, fat mass, fat-free mass, and percentage body fat, the largest increase in R^2 – apart from adjustments for sex and current height SDS – was observed with the input of early postnatal weight gain into the model. The percentages of variance explained by early postnatal and late infancy weight gain were larger for adult fat mass than for adult fat-free mass.

Table 2. Characteristics of participants at age 19 years by sex.

Characteristic	Men	Women	P
N	187	216	
Height			
- cm	179.4±7.9	166.4±7.1	0.001
- SDS	-0.55±1.1	-0.60±1.1	0.633
Weight			
- kg	69.9±12.1	60.5±10.6	0.001
- SDS	-0.41±1.2	-0.48±1.4	0.583
BMI			
- kg/m^2	21.7±3.1	21.8±3.4	0.659
- SDS	-0.10±1.2	-0.17±1.2	0.569
Waist circumference			
- cm	80.2±8.9	76.6±7.9	0.001
- SDS	0.24±1.1	0.73±0.92	0.001
Hip circumference			
- cm	92.1±8.1	94.2±9.4	0.017
- SDS	-0.22±1.2	0.03±1.1	0.037
Waist-to-hip ratio			
- cm/cm	0.87±0.054	0.82±0.063	0.001
- SDS	0.72±0.92	0.90±0.93	0.055
Sum of 4 skinfold thicknesses			
- mm	41.3±20.6	62.6±22.4	0.001
- SDS	1.7±2.8	1.1±1.6	0.012

Values represent mean±SD. Variables were compared with the unpaired t test.

Discussion

This study describes the results of a large-scale prospective study on the relation between birth weight, postnatal weight gain, and anthropometric parameters at the age of 19 years in subjects born very preterm and provides exclusive information about the predictive value of weight gain during the first 2 trimesters of pregnancy for adult body composition.

In our study, there might have been an interference of the effects of possible programming (i.e., the lifelong changes in structure or function of body systems that follow a specific insult in

early life) and the effects of prematurity on BMI and body composition in young adulthood. We studied only children with a gestational age <32 weeks and corrected birth weight for gestational age, which facilitated a valid comparison within the cohort. The results may not be generalizable to infants born at term but do provide useful information about fetal growth restriction in infants born very preterm. We did not separately address the effect of gestational age on adult outcomes, because this interesting issue provides sufficient data for a different study.

Inherent to the population studied, perinatal mortality was high, especially in those infants with a shorter gestational age and to a lesser extent in those with a lower absolute birth weight. However, no significant difference in birth weight SDS was found between those who died and those who survived; therefore, confounding by selective mortality seems unlikely. The same reasoning can be applied to the response and the non-response groups. Some subjects had missing data on weight at 3 months or 1 year, but these missing data were not related to any of the outcome measures.

We found some differences between anthropometric characteristics at age 19 years between the male and the female participants. Whereas the differences in absolute values were expec-

Table 3. Regression analyses of birth weight SDS and infancy weight gain on size and body composition at age 19 years.

Outcome	Birth weight SDS			Early postnatal weight gain			Late infancy weight gain		
	N	ß	95% CI	N	ß	95% CI	N	ß	95% CI
Height SDS	403	0.366	0.265 to 0.466	373	0.238	0.150 to 0.325	351	0.422	0.310 to 0.535
Height SDS adjusted for target height SDS	401	0.299	0.218 to 0.381	371	0.202	0.132 to 0.273	351	0.240	0.143 to 0.337
Weight SDS	403	0.369	0.248 to 0.489	373	0.321	0.215 to 0.427	351	0.445	0.311 to 0.580
BMI SDS	403	0.152	0.036 to 0.268	373	0.196	0.092 to 0.300	351	0.215	0.078 to 0.356
Waist circumference SDS	399	0.106	0.005 to 0.207	369	0.173	0.082 to 0.263	347	0.218	0.096 to 0.339
Waist circumference SDS adjusted for current height SDS	399	0.00546	-0.098 to 0.109	369	0.111	0.020 to 0.203	347	0.138	0.009 to 0.267
Hip circumference SDS	399	0.155	0.042 to 0.268	369	0.173	0.072 to 0.273	347	0.288	0.153 to 0.424
Hip circumference SDS adjusted for current height SDS	399	0.0208	-0.093 to 0.135	369	0.0879	-0.012 to 0.188	347	0.166	0.025 to 0.307
Sum of 4 skinfold thicknesses SDS	390	0.0535	-0.170 to 0.277	361	0.190	-0.015 to 0.394	340	0.286	0.011 to 0.561
Fat mass (kg)[a]	390	0.826	0.264 to 1.389	361	0.873	0.370 to 1.376	340	1.275	0.614 to 1.936
Fat mass (kg) adjusted for current height SDS[a]	390	0.331	-0.252 to 0.914	361	0.599	0.091 to 1.108	340	0.961	0.258 to 1.665
Fat-free mass (kg)[a]	390	2.181	1.582 to 2.779	361	1.639	1.116 to 2.161	340	2.429	1.178 to 3.081
Fat-free mass (kg) adjusted for current height SDS[a]	390	0.811	0.310 to 1.312	361	0.855	0.420 to 1.290	340	1.202	0.605 to 1.798
Percentage body fat (%)[a]	390	0.176	-0.329 to 0.682	361	0.479	0.022 to 0.936	340	0.651	0.033 to 1.269

Waist-to-hip ratio SDS, subscapular-to-triceps ratio, and the interaction terms between birth weight and infancy weight gain were not significant and thus were not reported.

[a] Adjusted for sex.

ted, the different SDSs for a few outcomes were not. However, because these sex differences were found in unplanned post hoc analyses, the results should be interpreted very cautiously. Adjustment for sex did not change the conclusions of the study.

To determine fat mass and distribution we used skinfold thicknesses, which are known to be prone to inter-observer variation (38). However, although skinfold-thickness measurements tend to overestimate fat mass somewhat compared with a direct method such as dual-energy X-ray absorptiometry, Fewtrell et al (39) concluded from their study on prematurity and body fatness at age 8 to 12 years that the same associations were found with both methods. The correlations between the anthropometric data of Durnin and dual-photon absorptiometry are 0.76 and 0.83 for males and females, respectively (40). A study of the reproducibility of the skinfold-thickness measurements used in the POPS-19 study showed that the reliability of the skinfold-thickness measurements was relatively low, but the reliability of the derived estimates of body composition was much higher (intraclass correlation coefficients ranged from 0.55 to 0.98), with a high intra-observer reliability (intraclass correlation coefficient >0.99). Because the birth weights of participants did not substantially differ between centers, this relatively low inter-observer reliability will have only attenuated the associations between birth weight and body composition at age 19 years.

We found that birth weight was positively associated with weight, height, and BMI at age 19 years. These findings are consistent with those of studies in populations born at term (11;12). Our study indicates that the positive association between birth weight and adult BMI is determined as early as in the first 2 trimesters of pregnancy. This finding conflicts with the results of the Dutch famine studies, which suggest that maternal malnourishment during early gestation predisposes to later obesity in the offspring (7;8). Our study does not confirm the J- or U-shape relationship between birth weight and adult BMI found in some studies (13;41;42),

Table 4. Explained variance (R^2) and change in explained variance (R^2 change) for the anthropometric variables at age 19 years.

Outcome	N	R^2 change						Total R^2
		Target height SDS	Adult height SDS	Sex	Birth weight SDS	Early postnatal weight gain	Late infancy weight gain	
Height SDS	351	37.5[a]	-	-	6.2[a]	4.5[a]	3.3[a]	51.6
Weight SDS	351	-	-	-	9.6[a]	9.6[a]	8.8[a]	28.1
BMI SDS	351	-	-	-	2.7[a]	4.9[a]	2.5[a]	10.1
Waist circumference SDS	347	-	8.8[a]	-	0.0	2.3[a]	1.1[a]	12.2
Sum of 4 skinfold thicknesses SDS	340	-	-	-	0.1	1.5	1.5[a]	3.1
Fat mass (kg)	340	-	5.4[a]	25.2[a]	0.2	1.8[a]	1.4[a]	34.1
Fat-free mass (kg)	340	-	18.1[a]	60.1[a]	0.7[a]	1.0[a]	0.9[a]	80.9
Percentage body fat (%)	340	-	-	64.7[a]	0.0	0.7[a]	0.4[a]	65.8

Values represent percent.
[a] Significant R^2 change ($P < 0.05$).

which might form a biological link between low birth weight and adult diseases. This suggests that either the associations mentioned above are established during the third trimester of pregnancy or that there is another link between fetal growth and adult disease. Singhal et al (18) proposed that this link might be formed by fat-free mass. However, though fat-free mass was significantly associated with birth weight, our data show no significant association between birth weight and percentage body fat in adulthood.

Although prenatal weight gain was not associated with percentage body fat, more early postnatal weight gain was associated with both a higher BMI and a higher percentage body fat at age 19 years. The higher BMI found agrees with the findings of earlier studies in which a positive association between early growth and adult BMI and obesity was found (13;43). Our study showed that this association was independent of birth weight and that the higher BMI was partly accounted for by a higher percentage body fat, at least in premature infants. So far, only a few studies have addressed the relationship between early growth and adult fat mass and distribution. From our results it may be concluded that the positive associations found by Ong et al (6) and Stettler et al (24) between early catch-up growth and fatness in childhood persist into young adulthood. This is in accordance with a study by Li et al (25) about early postnatal growth in length and adult fat-free mass in a Guatemalan population.

Moreover, we also found that a greater postnatal weight gain was associated with a higher adult waist circumference, both when adjusted and unadjusted for current height SDS. Fetal weight gain was also positively associated with waist circumference SDS, but after adjustment for current body height SDS, the association completely disappeared; this finding indicates that the increase in waist circumference with higher birth weight reflects mainly an increase in body size and not solely an increase in visceral fat. Prenatal and postnatal weight gain were not significantly associated with waist-to-hip ratio or subscapular-to-triceps ratio, although a tendency for low birth weight to be associated with a higher waist-to-hip ratio and a subscapular-to-triceps ratio was found. This finding agrees with the results of Fall et al (13) and Li et al (25). In some studies, low birth weight and early growth have been associated with a more truncal and abdominal fat pattern (13;16;17) but only after adjustment for current BMI. Although adjustment for current body size in "fetal origins" studies should always be interpreted cautiously, it might be arguable for some adult disease outcomes (44). However, we think it is theoretically not correct to adjust for current BMI – which includes current fat mass – in analyses with fat mass and fat distribution as outcomes. If correction for current body proportions is applied, an index independent of body fat should be used.

The associations found between birth weight, early postnatal, and late infancy weight gain, and adult BMI and body composition might be explained by perinatal programming (45). However, it is also possible that genes that influence prenatal, perinatal, and adult determinants underlie the associations found. More research is required about the possible mechanisms of programming of body proportions and body composition.

Conclusions

In conclusion, in individuals born very preterm both gestation, the period from birth until 3 months post-term, and the period from 3 months until 1 year post-term seem to be important predictors of body size and body mass in young adulthood. Greater weight gain during these periods is associated with higher height, weight, BMI, and fat-free mass at age 19 years. Birth weight in infants born very preterm is not associated with fat distribution. However, early postnatal weight gain and late infancy weight gain are – independently of birth weight or current height – associated with a more abdominal pattern of fat distribution and a higher percentage body fat. The relative effect of weight gain from birth until 3 months post-term on adult fat mass and fat distribution is more pronounced than is the effect of weight gain from 3 months until 1 year post-term.

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5 Preterm birth and lipid profile and carotid intima-media thickness in adulthood:

no effects of intrauterine or infancy weight gain

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Abstract

Objectives:

Cardiovascular disease (CVD) risk is associated with prenatal and infancy growth. However, the relative importance of these time periods for the CVD risk is uncertain. To elucidate this, we tested in a very preterm cohort (gestational age <32 weeks) the effects of birth weight for gestational age (as SD score, SDS), and weight gain between birth and 3 months post-term (early postnatal weight gain) and between 3 months and 1 year post-term (late infancy weight gain) on the lipid profile and carotid intima-media thickness (CIMT) at age 19 years.

Methods:

This was a prospective follow-up study in 364 subjects from the Project On Preterm and Small-for-gestational-age infants cohort, in whom fasting lipid levels and CIMT were measured at age 19 years.

Results:

A less favourable lipid profile was strongly associated with higher current BMI SDS, greater waist circumference SDS, and greater absolute fat mass. CIMT was positively associated with current height SDS, and with low-density lipoprotein (LDL) cholesterol and apolipoprotein B (ApoB) levels, and LDL/high-density lipoprotein cholesterol and apoB/apolipoprotein AI ratios. Lipid profile and CIMT were unrelated to gestational age, birth weight SDS, and early postnatal weight gain. CIMT was positively associated with late infancy weight gain, but the relationship disappeared after correction for current height SDS.

Conclusions:

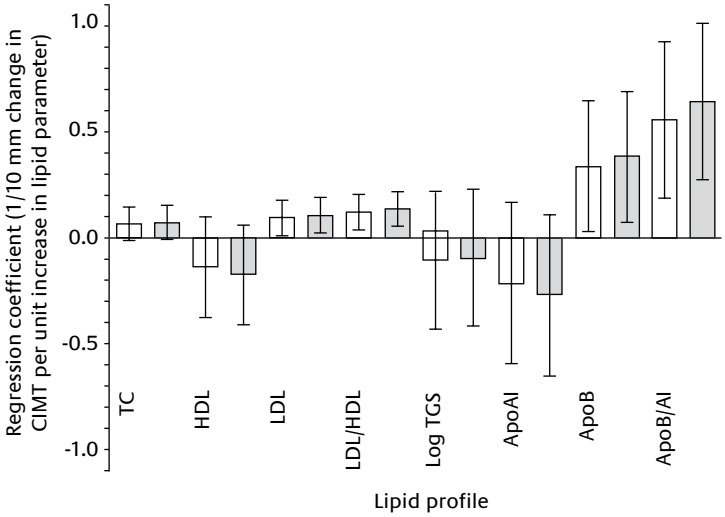
Our findings in 19-year-olds born very preterm argue for an effect of current body composition, rather than of early growth, on the CVD risk. Attempts to reduce the CVD risk in this specific population should focus on weight reduction in young adulthood rather than on optimizing the early growth pattern.

Introduction

The "fetal origins of adult disease" hypothesis postulates that cardiovascular disease (CVD) has its origin in early life, when specific insults during critical periods in development may permanently alter a body's structure and metabolism (1). Epidemiologic studies have provided evidence for this hypothesis by reporting an inverse association between birth weight and mortality from cardiovascular disease (2), non-fatal cardiovascular events (3), and the degree of carotid stenosis assessed by intima-media thickness (IMT) (4). Also, it has been demonstrated in elderly men that slow growth until the age of 1 year after low birth weight amplified risk of coronary artery disease (5). In contrast, others have shown in boys and girls aged 13 to 16 years who were born prematurely that rapid instead of slow weight gain in the first 2 postnatal weeks was associated with decreased brachial flow-mediated dilation (FMD), an early marker of the atherosclerotic process (6). Thus, in early life, at different stages of development, rapid growth and slow growth have been associated with increased CVD risk.

An atherogenic lipid profile is one of the major risk factors for CVD and is characterized by high levels of total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, triglycerides (TGS), and apolipoprotein B (ApoB), and low levels of high-density lipoprotein (HDL) cholesterol and apolipoprotein AI (ApoAI) (7). In a number of studies in individuals born after term gestation, birth weight has shown weak associations with some lipid levels (systematically reviewed in Lauren et al (8) and Huxley et al (9)).

Figure 1. Effect of the lipid profile on CIMT at age 19 years.



TC, HDL, LDL, and TGS in mmol/l. ApoAI and ApoB in g/l. All analyses were adjusted for gender and assessor. Grey columns represent analyses also adjusted for current height SDS.

As fetal weight gain reaches its maximum between 32 and 38 weeks of gestation, the circumstances in the third trimester of pregnancy contribute to most of the variation in birth weight. Detrimental circumstances resulting in intrauterine growth retardation can occur early and/or late in gestation. Persons who were in utero in the first trimester of pregnancy during the Dutch famine had the highest prevalence of coronary artery disease and also the highest LDL/HDL ratio (10;11), implying that insults during early gestation predispose to increased CVD risk. Whether lower birth weight for gestational age after the first 2 trimesters of pregnancy could explain increased CVD risk can only be answered by studies in subjects born very preterm (<32 gestational weeks).

We provide prospective long-term follow-up into adulthood of a well-described cohort of men and women born very preterm in whom lipid levels and carotid IMT (CIMT) were measured at the age of 19 years. Within this study population, we tested the relative importance of the effects

Table 1. Perinatal characteristics of participants.

Characteristic	Study sample with blood specimen only	Study sample with blood specimen and CIMT measurement
N	180	184
General		
Men (%)	47.8	46.7
White race (%)	88.3	88.4
Socio-economic status (1-6)[a]	3.4±1.5	3.6±1.6
Obstetric		
Maternal age (yrs)	27.3±5.4	26.7±5.8
Part of multiple pregnancy (%)	23.3	24.5
Hypertension during pregnancy (%)	15.6	19.0
Smoking during pregnancy (%)	33.3	26.7
Drugs and alchohol intoxication (%)[b]	51.7	50.0
Prolonged rupture of membranes (%)	22.2	22.8
Neonatal		
Gestational age (weeks)	29.8±1.5	29.8±1.4
Birth weight		
- g	1,335±331	1,330±341
- SDS	-0.08±1.02	-0.11±1.02
Weight at 3 mo		
- g	5,208±899	5,100±851
- SDS	-0.87±1.35	-1.00±1.31
Weight at 1 yr		
- g	8,991±1,242	8,846±1,144
- SDS	-0.91±1.19	-1.08±1.10

Values represent mean±SD or percent. Continuous variables were compared with the unpaired t test.
Dichotomous variables were compared by the χ^2 test.
[a] Mann-Whitney U test.
[b] Smoking, drinking alcohol, or using soft drugs, hard drugs or methadone during pregnancy.

of birth weight for gestational age and postnatal weight gain until the age of 1 year post-term on the lipid profile and CIMT in young adulthood. Weight at birth, and at the ages of 3 months and 1 year post-term had been recorded, so that the effects of weight gain before 32 weeks of gestation, between preterm birth and 3 months post-term (early postnatal weight gain), and between 3 months and 1 year post-term (late infancy weight gain) could be unravelled. In comparison, we tested the effect of indices of current fat mass/distribution on the lipid profile and CIMT.

Methods

Population

The Project On Preterm and Small-for-gestational-age infants (POPS) cohort study is a nationwide multicenter prospective follow-up study comprising 94% of all very preterm (<32 weeks' gestation) and/or very-low-birth-weight (<1,500 g) infants born in The Netherlands in 1983, in which birth, growth, and a number of other characteristics have been recorded from birth onwards (12;13). Gestational age (best obstetric estimate) was based upon last menstrual period, pregnancy testing, and ultrasound, if necessary.

At age 19 years, all 669 living subjects with a gestational age <32 weeks who were free from congenital skeletal deformations, Down's syndrome, chromosomal abnormalities, multiple congenital deformations, or inborn errors of metabolism were approached by mail to participate in the POPS-19 study. Subjects with diabetes or familial dyslipidemia or taking thyroid hormone or systemic corticosteroids were excluded. The approval of the medical ethical committees of all participating centers was obtained for the POPS-19 study.

Study protocol

Subjects who gave written informed consent to participate were seen after an overnight fast between 8.30 and 10.00 h between April 2002 and May 2003 at one of the outpatient clinics of the 10 participating centers. Assessors were blinded with respect to the perinatal characteristics of the subjects.

Venous blood was obtained after 30 minutes in supine position. Thereafter, CIMT was measured and anthropometry performed. For the latter, subjects were measured barefoot while wearing underclothing only. All 15 assessors had received extensive training before the start of the POPS-19 study and retraining during the entire study period every 2 months. Weight was measured to the nearest 0.1 kg on a balance scale, and height to the nearest 0.1 cm with a fixed stadiometer. Waist circumference was measured at 0.1-cm accuracy after gentle expiration, with a flexible tape measure. Four skinfold thickness measurements were taken in duplicate with a calibrated skinfold calliper on the left side of the body: in the triceps, biceps, subscapular, and iliacal regions. From these measurements, absolute fat mass was cal-

culated using the equation of Durnin and Ramahan (14). A more detailed description of skinfold thickness measurements obtained in the POPS-19 study has been published elsewhere (15).

Laboratory analysis

Blood samples were stored at -80 °C and thawed only once immediately before analysis. TC and TGS were measured in a fully automated computerized laboratory system with a Hitachi 747 (Hitachi, Tokyo, Japan). HDL, LDL, ApoAI, and ApoB were measured with a turbidimetric assay on a Hitachi 911.

Carotid intima-media thickness

Both left and right common carotid arteries (CCAs) were visualised in B mode using high-resolution devices with linear array transducers. Five assessors from 3 study centers had only devices with non-linear array transducers available for our study. Consequently, these assessors did not obtain images in the subjects who attended at their centers. The CCA was defined as the area 2 to 5 cm proximal from the beginning of the widening of the carotid bifurcation. For the left and right CCAs, 3 images were obtained in an optimal longitudinal direction and frozen on the R wave by electrocardiographic triggering and recorded on DICOM CD, Magneto Optical Disk or Super-VHS videotape.

Table 2. Characteristics of participants at age 19 years by sex.

Characteristic	Men	Women
N	172	192
Body size		
BMI SDS	-0.11±1.13	-0.19±1.25
Waist circumference SDS[b]	0.22±1.09	0.71±0.99
Absolute fat mass (kg)[b]	11.5±5.7	18.3±6.9
Lipid profile		
TC (mmol/l)	3.89±0.75[b]	4.45±0.84
HDL (mmol/l)	1.19±0.23[b]	1.44±0.32
LDL (mmol/l)	2.32±0.67[b]	2.63±0.76
LDL/HDL ratio	2.02±0.72	1.93±0.75
TGS (mmol/l)	0.86 (0.61 to 1.23)	0.80 (0.55 to 1.07)
ApoAI (g/l)	1.18±0.14[b]	1.37±0.20
ApoB (g/l)	0.67±0.18[b]	0.78±0.21
ApoB/AI ratio	0.57±0.16	0.58±0.17
CIMT[a]		
Near wall (mm)	0.53±0.07	0.54±0.07
Far wall (mm)	0.46±0.06	0.44±0.05
Mean of near + far wall (mm)	0.49±0.05	0.49±0.05

Values represent mean±SD or median (interquartile range). Variables were compared with the unpaired t test.
[a] Measurements in 86 men and 98 women.
[b] P <0.001 between men and women.

Images were sent to an independent core laboratory (Heart Core BV, Leiden, The Netherlands), where the off-line analysis was executed by 1 expert reader according to standard operating proceduces. For each reading, full quality control was performed by a second reader, and a third reader did the adjudication, if necessary. Readers were blinded with respect to the characteristics of the subjects. Quality of images was considered sufficient if at least the near or the far wall of the intima-media complex could be visualized and measured over a distance of at least 4.4 mm in a horizontal plane in combination with the appropriate calibration factor in agreement with the specifications of the validated software.

Super-VHS images were digitized first using the Vascular Imager v. 4.2.2. (Medical Image Applications, USA), and stored in RAW format at a resolution of 512 x 768 pixels with 256 bits of grey levels. DICOM CD and Magneto Optical Disk images were stored at the same resolution and grayscale. Subsequently, all images were calibrated and analyzed using the Carotid Analyzer v. 4.2.2. (Medical Image Applications). Thereafter, a digital region of interest was defined in the CCA. The maximum region of interest was selected within 2 to 5 cm proximal from the carotid bifurcation. Analysis was by automated border detection within the region of interest of the near and far wall.

Before the start of the POPS-19 study, assessors had received a 2-day training at the coordinating center (Leiden University Medical Center), and personal training at their own center. During the study, meetings between assessors were organized every 2 months, and reliability of CIMT measurements of the right CCA was tested at each center in a group of 4 healthy young adults. Two assessors from 1 study center failed to obtain images of sufficient quality. Their images (of 41 subjects) were excluded from the statistical analysis. Of the remaining 8 assessors, the inter-observer coefficient of variation was 6.3%.

Statistical analysis

Measurements of size at birth and on subsequent occasions were converted to SD score (SDS), using Swedish references for preterm infants (16) and recently collected Dutch references (17-19), respectively. An exception was made for fat mass because of lack of population references. Early postnatal weight gain was calculated by subtracting birth weight SDS from the weight at 3 months SDS. Late infancy weight gain was calculated by subtracting weight at 3 months SDS from weight at 1 year SDS.

Linear regression analysis was used to assess the effects of gestational age, early growth, and current size and body composition on the lipid profile and CIMT at age 19 years. To differentiate the effects of birth weight SDS, early postnatal weight gain, and late infancy weight gain on these outcomes, early postnatal weight gain was adjusted for the effect of birth weight SDS, and late infancy weight gain for the effects of both birth weight SDS and early postnatal weight gain in multivariate regression models, consistent with previous analyses in this cohort (15). Not normally distributed outcomes (TGS only) were ^{10}log-transformed before statistical comparison.

All analyses were adjusted for gender, and analyses with CIMT also for assessor. Analyses with early growth were repeated with adjustment for the possible confounders race (white or non-white), socio-economic status (≤ or >2; on a 6-point scale, in which 1 was lowest and 6 highest), multiple pregnancy (singleton or non-singleton), and gestational age (as continuous as well as dichotomous (≤ or >30 weeks) variable).

Results

At age 19 years, 669 men and women born before 32 weeks of gestation were still alive and eligible for inclusion: 415 consented to participate, and 254 refused or could not be traced. Three of the 415 participants met one of the exclusion criteria (strong suspicion of familial dyslipidemia by 1 person and systemic corticosteroid use by 2 subjects), 25 failed to give blood, 23 attended without fasting, and from 180 no (satisfactory) measurement of CIMT was available. Therefore, 364 and 184 individuals were analyzed with respect to the lipid profile and CIMT, respectively.

There were no significant differences in perinatal characteristics between subjects with and those without CIMT measurement (Table 1). In both groups, there was a slight female

Table 3. Regression analyses of adult size and body composition on the lipid profile and CIMT at age 19 years.

Outcome	Height SDS			BMI SDS			Waist circumference SDS			Absolute fat mass (kg)		
	N	ß	95% CI	N	ß	95% CI	N	ß	95% CI	N	ß	95% CI
TC (mmol/l)	357	-0.060	-0.136 to 0.034	356	0.153	0.086 to 0.015	357	0.145	0.065 to 0.221	353	0.021	0.008 to 0.224
HDL (mmol/l)	357	0.002	-0.025 to 0.029	356	-0.050	-0.074 to -0.026	357	-0.067	-0.095 to -0.039	353	-0.009	-0.013 to -0.004
LDL (mmol/l)	356	-0.054	-0.122 to 0.014	355	0.162	0.102 to 0.222	356	0.164	0.093 to 0.234	352	0.024	0.012 to 0.035
LDL/HDL ratio	356	-0.034	-0.104 to 0.036	355	0.207	0.146 to 0.268	356	0.242	0.172 to 0.312	352	0.033	0.022 to 0.045
Log TGS (mmol/l)	356	-0.005	-0.024 to 0.015	355	0.047	0.030 to 0.065	356	0.055	0.035 to 0.075	352	0.007	0.003 to 0.010
ApoAI (g/l)	356	0.009	-0.008 to 0.026	355	-0.008	-0.023 to 0.008	356	-0.011	-0.029 to 0.006	352	-0.003	-0.006 to 0
ApoB (g/l)	356	-0.015	-0.033 to 0.004	355	0.049	0.032 to 0.065	356	0.049	0.029 to 0.068	352	0.006	0.003 to 0.010
ApoB/AI ratio	356	-0.014	-0.030 to 0.002	355	0.043	0.030 to 0.057	356	0.046	0.030 to 0.062	352	0.006	0.004 to 0.009
CIMT (10^{-1} mm)[a]	183	0.071	0.011 to 0.130	182	0.013	-0.046 to 0.071	183	0.044	-0.018 to 0.106	182	0.005	-0.006 to 0.015

All analyses were adjusted for sex.

[a] Mean of near + far wall adjusted for assessor.

preponderance. Mean birth weight for gestational age was equal to the population reference mean, whereas weight at 3 months and 1 year was approximately 1 SD below the population mean. The value for weight at 3 months was missing for 25 subjects and the value for weight at 1 year was missing for 29.

At age 19 years, women had a greater waist circumference SDS and greater absolute fat mass than men (Table 2). Also, they had higher levels of nearly all lipids. TGS and CIMT were not different between genders.

TC, LDL, TGS, and ApoB levels and LDL/HDL and ApoB/AI ratios were each positively associated with current BMI SDS, waist circumference SDS, and absolute fat mass (Table 3). HDL level was inversely associated with all of these indices. ApoAI level was inversely associated only with absolute fat mass. CIMT was positively related to current height SDS. Therefore, further analyses with CIMT were also adjusted for current height SDS.

LDL and ApoB levels and LDL/HDL and ApoB/AI ratios were positively associated with CIMT (Figure 1). After adjustment for current height SDS, the observed relations became somewhat stronger.

The lipid profile was unrelated to gestational age, birth weight SDS, early postnatal weight gain, and late infancy weight gain (Table 4). Adjustment for race, socio-economic status, multiple pregnancy, and gestational age (as continuous or dichotomous variable in the analyses with early growth) did not change these associations (data not shown). There was no evidence for interaction between birth weight SDS and gestational age on the lipid profile (data not shown).

CIMT was positively associated with late infancy weight gain: 0.091 (95% CI: 0.005 to 0.178) x 10^{-1} mm per 1 SDS increase in weight gain (Table 4). After adjustment for current height SDS, the association lost statistical significance. Adjustment for the same possible confounders as above slightly reduced the strength of these associations (data not shown).

Discussion

We studied the lipid profile and CIMT in relation to early growth and current body composition in young adults born very preterm who had been followed prospectively since birth. Our findings argue for an effect of current body composition, rather than of early growth, on the CVD risk in this specific population.

Although weak associations between birth weight and some lipid levels have been reported in full-term individuals at different ages (8;9), we could not confirm such relations in our population of 19-year-olds born very preterm. Instead, we found that greater current absolute fat mass and a more central pattern of fat distribution were strongly related to a less favourable lipid profile. Also, we found that LDL and ApoB levels and LDL/HDL and ApoB/AI ratios were positively related to CIMT, while all lipid levels were within the normal range. We found

Table 4. Regression analyses of gestational age, and birth weight SDS and infancy weight gain on the lipid profile and CIMT at age 19 years.

Outcome	Gestational age (weeks)			Birth weight SDS			Early postnatal weight gain			Late infancy weight gain		
	N	ß	95% CI	N	ß	95% CI	N	ß	95% CI	N	ß	95% CI
TC (mmol/l)	364	0.037	-0.018 to 0.093	364	-0.013	-0.094 to 0.068	339	0.055	-0.018 to 0.129	319	-0.049	-0.149 to 0.051
HDL (mmol/l)	364	0.005	-0.015 to 0.025	364	-0.015	-0.043 to 0.014	339	0.005	-0.021 to 0.032	319	-0.020	-0.057 to 0.016
LDL (mmol/l)	363	0.031	-0.019 to 0.081	363	-0.001	-0.073 to 0.072	338	0.056	-0.009 to 0.121	318	-0.021	-0.110 to 0.068
LDL/HDL ratio	363	0.011	-0.041 to 0.062	363	0.015	-0.060 to 0.090	338	0.023	-0.043 to 0.090	318	0.022	-0.071 to 0.114
Log TGS (mmol/l)	363	0.004	-0.011 to 0.018	363	0.005	-0.016 to 0.026	338	-0.002	-0.021 to 0.017	318	-0.014	-0.041 to 0.013
ApoAI (g/l)	363	0.008	-0.004 to 0.020	363	-0.005	-0.023 to 0.013	338	0.012	-0.004 to 0.028	318	-0.013	-0.036 to 0.009
ApoB (g/l)	363	0.011	-0.003 to 0.024	363	-0.001	-0.021 to 0.019	338	0.015	-0.003 to 0.033	318	-0.010	-0.035 to 0.014
ApoB/AI ratio	363	0.005	-0.007 to 0.016	363	0	-0.017 to 0.017	338	0.004	-0.011 to 0.019	318	0	-0.021 to 0.020
CIMT (10^{-1} mm) [a]	184	-0.004	-0.050 to 0.042	184	-0.008	-0.073 to 0.057	171	0.019	-0.042 to 0.080	160	0.091	0.005 to 0.178
CIMT adjusted for current height SDS (10^{-1} mm) [a]	183	-0.004	-0.050 to 0.042	183	-0.047	-0.117 to 0.022	170	0.002	-0.059 to 0.063	159	0.053	-0.039 to 0.145

All analyses were adjusted for sex.

[a] Mean of near + far wall adjusted for assessor.

no direct effects of current body composition on CIMT at age 19 years, but given the effects of current body composition on the lipid profile and of the lipid profile on its turn on CIMT, such effects are to be expected with advancing age.

Our observation that rapid weight gain in the period between 3 months and 1 year was associated with marginally increased CIMT at age 19 years is in contrast with findings from others in eldery men that slow growth until 1 years of age after low birth weight was associated with increased coronary artery disease risk (5). Recently, in prematurely born boys and girls aged 13 to 16 years, it was found that rapid instead of slow weight gain in the period between birth and 2 weeks post-partum, during which an individual is normally still in utero, was associated with decreased brachial FMD (6). However, in that study, the effect of weight gain after hospital discharge on FMD had not been taken into consideration.

The observed effect of rapid late infancy weight gain on increased CIMT in our study may be causal, or, alternatively, it may be partly explained by confounding because late infancy weight gain was positively associated with current height SDS (15) and current height SDS with CIMT. Indeed, the relation between late infancy weight gain and CIMT disappeared after cor-

rection for current height SDS. It is unclear whether the loss of association was due to (appropriate) correction for a confounding variable or (inappropriate) correction for an intermediate variable in the causal pathway. However, we did not find an effect of late infancy weight gain on the lipid profile, thereby making a possible biologic basis for the effect of rapid late infancy weight gain on increased CVD risk less likely.

CIMT is a measure of structural atherosclerosis, which corresponds well with the true histological thickness (20;21). It is a strong predictor of stroke and myocardial infarction (22;23). Because in our study ultrasound images were acquired by multiple assessors (N=8), associations of CIMT with early growth may have been missed due to lack of precision. In our subjects aged only 19 years, CIMT was positively related to LDL and ApoB levels and LDL/HDL and ApoB/AI ratios, whereas all lipid levels were within the normal range. This suggests that CIMT has been measured reliably enough to detect statistically significant relationships with well-known risk factors for the atherosclerotic process. With a coefficient of variation of 6.3%, the inter-observer reproducibility of CIMT measurements was similar to studies involving only 2 to 3 assessors (24-26). Thus, in our study, associations of CIMT at age 19 years with early growth were not observed, simply because they were not there.

As we had incomplete follow-up data, it may be argued that the observed associations could be biased by non-response. Participants did not differ substantially from non-responders in perinatal characteristics, nor did participants who had their CIMT measured differ from those without CIMT measurement. Non-response in the POPS-19 study was associated only with male gender, non-white race, and lower socio-economic status (15). Men had lower levels of all lipids, except for TGS. Whites and non-whites had comparable lipid profiles. Lower socio-economic status was associated with a less favourable lipid profile (data not shown). However, none of these demographic factors had any effect on early growth, implying that non-response bias could not have substantially influenced our results.

Conclusions

In conclusion, our findings in 19-year-olds born very preterm argue for an effect of current body composition, rather than of early growth, on the CVD risk. Attempts to reduce the CVD risk in this specific population should focus on weight reduction in young adulthood rather than on optimizing the early growth pattern.

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6 Preterm birth and insulin resistance in adulthood:

higher fat mass after poor intrauterine weight gain has larger effects on insulin resistance than does higher fat mass after normal intrauterine weight gain

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Abstract

Objectives:

An increased risk of type 2 diabetes is associated with low birth weight after term gestation, including amplification of this risk by weight gain during infancy and adult body composition. Premature birth is also associated with insulin resistance, but studies conducted so far have not provided follow-up into adulthood. We studied the effects of lower birth weight (as SD score, SDS) and infancy weight gain on insulin resistance in 19-year-olds born before 32 weeks of gestation, and the interaction between lower birth weight SDS and infancy weight gain, as well as between lower birth weight and adult body composition, on insulin resistance.

Methods:

This was a prospective follow-up study in 346 subjects from the Project On Preterm and Small-for-gestational-age infants cohort, in whom fasting glucose, insulin, and C-peptide levels were measured at 19 years. Insulin resistance was calculated with homeostatic modelling (homeostatic model assessment for insulin resistance index, HOMA-IR).

Results:

Birth weight SDS was unrelated to the outcomes. Rapid infancy weight gain until 3 months post-term was weakly associated with higher insulin level (P=0.05). Adult fatness was positively associated with insulin and C-peptide levels and HOMA-IR (all P <0.001). On these parameters, there was a statistical interaction between birth weight SDS and adult fat mass (P=0.002 to 0.03).

Conclusions:

In subjects born very preterm, rapid infancy weight gain until 3 months predicted higher insulin levels at 19 years, but the association was weak. Adult obesity strongly predicted higher insulin and C-peptide levels as well as HOMA-IR. The effect of adult fat mass on these parameters was dependent on its interaction with birth weight SDS.

Introduction

Effects of intrauterine and postnatal growth on the risk, in the general population, of developing type 2 diabetes are well described. Low birth weight after term gestation is associated with insulin resistance, glucose intolerance, and type 2 diabetes in later life (1;2). The effect of low birth weight on increased type 2 diabetes risk is stronger in subjects who catch up in weight during infancy, and in those who become overweight during childhood and in adult life (3-5). Also, low weight in infancy has been associated with type 2 diabetes (1;6).

Less is known about the effects of intrauterine and postnatal growth on insulin resistance in the growing population of survivors of preterm birth. Recently, it was found that 7-year-old children born prematurely were more insulin-resistant than age-matched normal controls (7). The effect of prematurity was irrespective of intrauterine growth, although another study in 6-year-old preterm offspring found higher basal insulin and C-peptide levels in subjects with birth weights below the 10th percentile (8). However, both studies were performed in small populations. Very preterm (i.e., <32 weeks of gestation) infants differ from term children in postnatal growth pattern, which is characterized by an initial slowing of growth followed by late catch-up growth (9;10). To date, 1 study has focused on insulin resistance in relation to postnatal weight gain after preterm birth (11). The investigators found that insulin split products were higher in subjects aged 13 to 16 years with rapid weight gain in the first 2 weeks postnatally. As these 3 studies were conducted in paediatric populations, it remains uncertain whether the observed associations in childhood persist into adult life. For the same reason, it is unknown whether there is interaction between low birth weight and adult body composition on insulin resistance after very preterm birth.

We provide here a prospective long-term follow-up into adulthood of a well-described cohort of men and women born very preterm, in whom insulin resistance was assessed at the age of 19 years. Within this study population, we tested the effects of lower birth weight for gestational age and rapid infancy weight gain on insulin resistance at the age of 19 years. We also tested whether there was interaction between lower birth weight and rapid infancy weight gain, and between lower birth weight and adult body composition, on insulin resistance.

Methods

Population

The Project On Preterm and Small-for-gestational-age infants (POPS) study is a nationwide multicenter prospective follow-up study, which comprises 94% of all liveborn very preterm (<32 weeks of gestation) and/or very-low-birth-weight (<1,500 g) infants born in The Netherlands in 1983, and has documented birth, growth, and a number of other characteristics from birth

onwards (12;13). At follow-up visits at age 3 months and 1 year post-term, weight was recorded. At age 19 years, all 637 alive subjects born with a gestational age <32 weeks who were free from congenital skeletal deformations, Down's syndrome, chromosomal abnormalities, multiple congenital deformations or inborn errors of metabolism, and who were not born to mothers with gestational diabetes, were approached by mail to participate in the POPS-19 study. Subjects with diabetes mellitus, or on thyroid hormone or systemic corticosteroids, as well as pregnant women, were excluded. The approval of the medical ethical committees of all participating centres was obtained for the POPS-19 study.

Study protocol

Subjects who gave written informed consent to participate were seen after an overnight fast between 8.30 and 10.00 h between April 2002 and May 2003 at one of the outpatient clinics of the 10 participating centres. Assessors were blinded with respect to the perinatal characteristics of the subjects.

Table 1. Perinatal characteristics of participants and non-responders.

Characteristic	Participants	Non-responders	P
N	346	242	
General			
Males (%)	47.4	66.1	<0.001
Whites (%)	88.3	80.1	0.006
Socio-economic status (1-6)	3.5±1.6	2.8±1.5	<0.001 [a]
Obstetric			
Maternal age (yrs)	26.9±5.7	26.4±5.3	0.23
Parity >0 (%)	46.7	50.6	0.35
Part of multiple pregnancy (%)	24.3	21.5	0.43
Hypertension during pregnancy (%)	16.5	15.3	0.70
Smoking during pregnancy (%)	30.0	31.8	0.67
Drugs and alcohol intoxication (%) [b]	50.9	51.2	0.93
Prolonged rupture of membranes (%)	24.0	24.4	0.91
Neonatal			
Gestational age (weeks)	29.8±1.5	29.8±1.5	0.55
Birth weight			
- g	1,332±333	1,354±278	0.39
- SDS	-0.09±1.02	-0.08±0.90	0.98
Weight at 3 mo			
- g	5,169±871	5,234±896	0.40
- SDS	-0.92±1.32	-0.94±1.42	0.88
Weight at 1 yr			
- g	8,909±119	9,084±140	0.14
- SDS	-1.00±1.15	-0.95±1.38	0.61

Values represent mean±SD or percent. Continuous variables were compared with the unpaired t test.
Dichotomous variables were compared by the χ^2 test.
[a] Mann-Whitney U test.
[b] Smoking, drinking alcohol, or using soft drugs, hard drugs or methadone during pregnancy

Venous blood was obtained after 30 minutes in a supine position. Thereafter, anthropometry was performed, for which assessors had received extensive training prior to the study, and re-training during the entire study period at 2-month intervals. Subjects were measured barefoot while wearing underclothing only. Weight was measured to the nearest 0.1 kg on a balance scale, and height to the nearest 0.1 cm with a fixed stadiometer. Waist and hip circumferences were measured at 0.1-cm accuracy using standard methods (14). Four skinfold thickness measurements were taken in duplicate with a calibrated skinfold calliper on the left side of the body: at triceps, biceps, subscapular, and iliacal regions. From these measurements, fat mass and the corresponding fat-free mass were calculated using the equations of Durnin and Rahaman (15). A more detailed description of skinfold thickness measurements obtained in the POPS-19 study has been published elsewhere (16).

Laboratory analysis

Blood samples were stored at -80 °C and thawed only once immediately before analysis. Glucose was measured in a fully automated computerized laboratory system with an Hitachi 747 (Hitachi, Tokyo, Japan) chemistry analyser, and insulin and C-peptide were measured with highly sensitive radioimmunoassays (Linco, St Charles, MO, USA; detection levels 0.1 mU/l

Table 2. Characteristics of participants at age 19 years by sex.

Characteristic	Men	Women	P
N	164	182	
Height			
- cm	179.4±7.7	166.4±7.3	<0.001
- SDS	-0.54±1.08	-0.60±1.13	0.66
Weight			
- kg	69.1±10.7	60.2±10.7	<0.001
- SDS	-0.47±1.12	-0.50±1.39	0.83
BMI			
- kg/m^2	21.4±2.8	21.7±3.2	0.44
- SDS	-0.17±1.10	-0.19±1.24	0.86
Waist circumference			
- cm	79.8±7.9	76.8±8.7	0.001
- SDS	0.19±1.04	0.70±0.98	<0.001
WHR			
- cm/cm	0.87±0.06	0.82±0.08	<0.001
- SDS	0.70±0.92	0.86±0.97	0.12
Absolute fat mass (kg)	11.1±5.2	18.2±6.6	<0.001
Fat-free mass (kg)	57.9±7.3	42.1±6.6	<0.001
Fat percentage (%)	15.7±5.3	29.5±7.1	<0.001
Glucose (mmol/l)	5.2±0.4	4.8±0.4	<0.001
Insulin (mU/l)	8 (6 to 11)	8 (7 to 11)	0.42
C-peptide (mmol/l)	0.66±0.23	0.69±0.21	0.16
HOMA-IR	1.9 (1.4 to 2.6)	1.8 (1.4 to 2.3)	0.46

Values represent mean±SD or median (interquartile range). Variables were compared with the unpaired t test.

and 0.03 mmol/l, respectively; interassay coefficient of variation 4.7-12.2% and 3.2-9.3% at different levels, respectively). A homeostatic model assessment for insulin resistance index (HOMA-IR) was calculated (17). Insulin and C-peptide levels, and HOMA-IR were considered as parameters of insulin resistance. Insulin level and HOMA-IR correlate strongly with Si assessed by the frequently-sampled intravenous glucose tolerance test in young persons (18;19).

Statistical analysis

Auxological data at birth and on subsequent occasions were converted to SD score (SDS), to correct for (gestational) age and sex, using Swedish references for preterm infants (20), and recently collected Dutch references (14;21;22), respectively.

Results in Tables 1 and 2 are presented as mean±SD, or median (interquartile range) if variables were not normally distributed (insulin and HOMA-IR). These variables were ^{10}log-transformed before statistical comparison.

Table 3. Regression analyses of birth weight SDS and infancy weight gain on the parameters of insulin resistance at age 19 years.

Outcome	Crude[a]			Adjusted[b]			Interaction with birth weight SDS		
	N	ß (95% CI)	P	N	ß (95% CI)	P	N	ß (95% CI)	P
Log Insulin									
- Birth weight SDS	346	-0.006 (-0.025 to 0.012)	0.49	339	-0.002 (-0.024 to 0.020)	0.86	-	-	-
- Weight SDS at 3 mo	321	0.017 (0.001 to 0.033)	0.04	315	0.018 (0 to 0.036)	0.05	321	-0.015 (-0.034 to 0.03)	0.10
- Weight SDS at 1 yr	317	0.016 (-0.002 to 0.035)	0.08	313	0.014 (-0.005 to 0.033)	0.15	317	-0.006 (-0.025 to 0.013)	0.53
C-peptide									
- Birth weight SDS	346	-0.020 (-0.043 to 0.002)	0.08	339	-0.020 (-0.043 to 0.003)	0.08	-	-	-
- Weight SDS at 3 mo	321	-0.010 (-0.030 to 0.011)	0.36	315	-0.006 (-0.029 to 0.017)	0.61	321	0.011 (-0.012 to 0.035)	0.34
- Weight SDS at 1 yr	317	-0.003 (-0.026 to 0.019)	0.78	313	-0.003 (-0.027 to 0.021)	0.81	317	0.004 (-0.018 to 0.027)	0.70
Log HOMA-IR									
- Birth weight SDS	345	-0.005 (-0.025 to 0.015)	0.65	338	0 (-0.23 to 0.23)	1.00	-	-	-
- Weight SDS at 3 mo	320	0.017 (0 to 0.035)	0.05	314	0.017 (-0.003 to 0.036)	0.09	320	-0.011 (-0.031 to 0.009)	0.27
- Weight SDS at 1 yr	316	0.020 (0 to 0.040)	0.05	312	0.017 (-0.004 to 0.037)	0.12	316	-0.003 (-0.023 to 0.017)	0.79

[a] Adjusted only for sex.

[b] Adjusted for sex, race (white or non-white), socio-economic status (≤ or >2), multiple pregnancy (singleton or non-singleton), gestational age (≤ or >30 weeks), parity (0 or >0), and hypertension during pregnancy (yes or no).

Birth weight is a strong predictor of postnatal size. Therefore, the multivariate linear regression model developed by Li et al was used to distinguish between the separate effects of birth weight SDS, and of postnatal size (at the age of 3 months, 1 year, and 19 years) on the parameters of insulin resistance (23). First, the effect of birth weight SDS on the parameters of insulin resistance was studied. Subsequently, residual (observed - expected) postnatal size was entered into the model. Expected postnatal size was based upon birth weight SDS only. Hence, residual postnatal size can be interpreted as growing more (or less) than would be expected from a given birth weight SDS. Thereafter, the interaction term (birth weight SDS x residual, with subtraction of means) was entered. Recently (24), the algebraic concept of this model was explained, showing that it can be rewritten to the model by Lucas et al (25). In the applied model, postnatal size is made statistically unrelated to birth weight SDS.

Analyses with birth weight SDS and infancy weight gain were repeated with adjustment for the possible confounders sex, race (white or non-white), socio-economic status (≤ or >2), multiple pregnancy (singleton or non-singleton), gestational age (≤ or >30 weeks), parity (0 or >0), and hypertension during pregnancy (yes or no). Analyses with adult size and body composition were repeated with adjustment for sex, race, and socio-economic status.

Statistical significance was defined as a P value ≤0.05. Non-linear associations were tested by first producing quarters of birth weight SDS and infancy weight gain. These quarters were compared with respect to the parameters of insulin resistance.

Results

At age 19 years, 637 men and women born before 32 weeks of gestation were still alive and eligible for inclusion: 395 consented to participate, whereas 242 refused or were not traceable. Three of the 395 participants met one of the exclusion criteria (1 woman was pregnant at the time of assessment, and 2 subjects used systemic corticosteroids), 27 failed to give blood, and 19 attended not-fasted. Therefore, 346 individuals were included in the statistical analyses.

Non-response was associated with male sex, non-white race, and lower socio-economic status (Table 1). It was not associated with gestational age or birth weight. Nineteen individuals (6%) had a birth weight <-2 SDS, 73 (23%) had a weight at 3 months <-2 SDS, and 57 (18%) had a weight at 1 year <-2 SDS.

For both sexes, mean values for height and weight were below the population reference means, while the means for waist circumference and waist-to-hip ratio were greater, especially in the women (Table 2). Women had greater absolute and relative fat mass than men. Men had greater fat-free mass and higher glucose levels than women. There was no difference between the sexes for insulin and C-peptide levels and HOMA-IR. Of the women, 121 (67%) used

oral contraceptives at the time of assessment, but there were no significant differences in the parameters of insulin resistance between pill and non-pill users (data not shown).

Birth weight SDS was unrelated to the parameters of insulin resistance (Table 3). Rapid infancy weight gain until 3 months was associated with a higher insulin level and HOMA-IR. After correction for possible confounders, the strength of the relationship with HOMA-IR remained unchanged but statistical significance was lost. Rapid infancy weight gain until 1 year was associated only with higher HOMA-IR, which relationship lost statistical significance after correction for possible confounders. No interaction between birth weight SDS and infancy weight gain on the parameters of insulin resistance was observed. There was no evidence for non-linearity in the associations between early growth and the parameters of insulin resistance (data not shown).

Except for height SDS, all measures of adult size and body composition were strongly associated with insulin and C-peptide levels as well as HOMA-IR (Table 4). Interaction between birth weight SDS and adult absolute and relative fat mass on these parameters was observed. Also, interaction between birth weight SDS and adult height SDS on insulin level and HOMA-IR, and between birth weight SDS and adult waist circumference SDS on HOMA-IR, was found. Figure 1 displays the direction of the interaction of birth weight SDS and adult absolute fat mass on HOMA-IR, showing that higher fat mass after lower birth weight SDS has larger effects on the parameters of insulin resistance than does higher fat mass after higher birth weight SDS.

Discussion

From birth onwards we followed a relatively large cohort of subjects born very preterm. At age 19 years, insulin resistance was assessed using fasting levels of insulin and C-peptide as well as HOMA-IR. We found that rapid infancy weight gain until 3 months predicted higher insulin levels at age 19 years, but the association was weak. Adult fat accumulation strongly predicted higher insulin and C-peptide levels as well as higher HOMA-IR at age 19 years. The effect of adult fat accumulation on these parameters of insulin resistance was dependent on its interaction with birth weight SDS. This was most clearly the case for increased fat mass per se, rather than for more abdominal fat. However, it should be emphasized that the typical centralization of fat distribution occurs with advancing age so that such relationships may become more clear later in adulthood.

It has been suggested that the association between early growth and type 2 diabetes risk is the result of a biological phenomenon which has been called fetal or perinatal programming (26). Throughout the years, several hypotheses based on the concept of programming have been proposed to underlie this association, including the “thrifty phenotype”, “fetal salvage”, “catch-up growth”, and “stem-cell” hypotheses (27-30). There are also indications that the as-

sociation is not the result of programming but of confounding (especially by socio-economic status), selective survival or response (31;32), or genes that affect both the path of early growth and insulin resistance (33). Despite incomplete follow-up, our participants did not significantly

Table 4. Regression analyses of adult size and body composition on the parameters of insulin resistance at age 19 years.

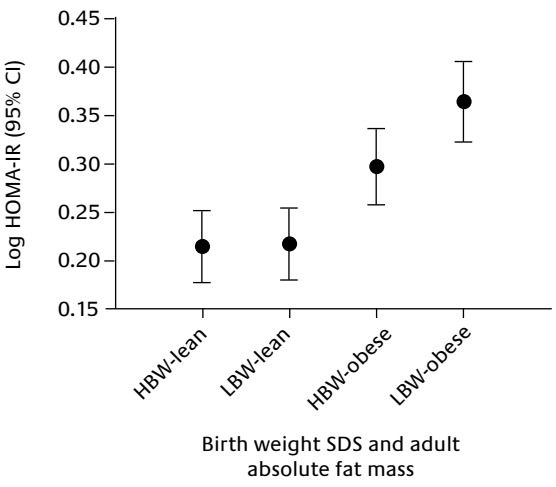
Outcome	Crude[a]			Adjusted[b]			Interaction with birth weight SDS		
	N	ß (95% CI)	P	N	ß (95% CI)	P	N	ß (95% CI)	P
Log Insulin									
- Height SDS	339	0.004 (-0.015 to 0.022)	0.70	333	0.001 (-0.018 to 0.020)	0.93	339	-0.022 (-0.040 to -0.005)	0.01
- BMI SDS	338	0.060 (0.045 to 0.075)	<0.001	332	0.057 (0.042 to 0.072)	<0.001	338	-0.012 (-0.026 to 0.002)	0.09
- Waist circumference SDS	341	0.076 (0.059 to 0.093)	<0.001	335	0.073 (0.056 to 0.091)	<0.001	341	-0.016 (-0.032 to 0)	0.06
- Waist-to-hip ratio SDS	340	0.030 (0.010 to 0.049)	0.003	334	0.045 (0.023 to 0.066)	<0.001	340	-0.007 (-0.026 to 0.012)	0.48
- Absolute fat mass (kg)	335	0.011 (0.008 to 0.014)	<0.001	329	0.011 (0.008 to 0.014)	<0.001	335	-0.005 (-0.009 to -0.002)	0.003
- Fat percentage (%)	337	0.008 (0.005 to 0.011)	<0.001	331	0.009 (0.006 to 0.012)	<0.001	337	-0.004 (-0.007 to 0)	0.03
C-peptide									
- Height SDS	339	0.016 (-0.007 to 0.038)	0.18	333	0.011 (-0.012 to 0.035)	0.34	339	0.001 (-0.020 to 0.022)	0.92
- BMI SDS	338	0.056 (0.037 to 0.075)	<0.001	332	0.055 (0.035 to 0.074)	<0.001	338	-0.017 (-0.035 to 0.001)	0.06
- Waist circumference SDS	341	0.084 (0.063 to 0.106)	<0.001	335	0.083 (0.061 to 0.105)	<0.001	341	-0.012 (-0.032 to 0.008)	0.25
- Waist-to-hip ratio SDS	340	0.045 (0.021 to 0.069)	<0.001	334	0.056 (0.030 to 0.082)	<0.001	340	-0.019 (-0.042 to 0.004)	0.11
- Absolute fat mass (kg)	335	0.016 (0.012 to 0.019)	<0.001	329	0.016 (0.012 to 0.019)	<0.001	335	-0.006 (-0.010 to -0.002)	0.006
- Fat percentage (%)	337	0.013 (0.010 to 0.017)	<0.001	331	0.014 (0.010 to 0.017)	<0.001	337	-0.004 (-0.008 to 0)	0.03
Log HOMA-IR									
- Height SDS	338	0.004 (-0.016 to 0.024)	0.70	332	0.001 (-0.020 to 0.021)	0.93	338	-0.023 (-0.042 to -0.004)	0.02
- BMI SDS	337	0.065 (0.050 to 0.081)	<0.001	331	0.063 (0.047 to 0.079)	<0.001	337	-0.013 (-0.028 to 0.002)	0.09
- Waist circumference SDS	340	0.083 (0.065 to 0.101)	<0.001	334	0.081 (0.062 to 0.099)	<0.001	340	-0.019 (-0.036 to -0.001)	0.04
- Waist-to-hip ratio SDS	339	0.034 (0.013 to 0.055)	0.002	333	0.047 (0.024 to 0.070)	<0.001	339	-0.007 (-0.028 to 0.014)	0.50
- Absolute fat mass (kg)	334	0.012 (0.009 to 0.015)	<0.001	328	0.013 (0.010 to 0.016)	<0.001	334	-0.006 (-0.009 to -0.002)	0.002
- Fat percentage (%)	336	0.009 (0.006 to 0.012)	<0.001	330	0.010 (0.007 to 0.013)	<0.001	336	-0.004 (-0.008 to -0.001)	0.01

[a] Adjusted only for sex.

[b] Adjusted for sex, race (white or non-white), and socio-economic status (≤ or >2).

differ from non-responders in perinatal characteristics. Non-response was higher among men, non-whites, and those with lower socio-economic status. As expected, we found that subjects with lower socio-economic status had higher parameters of insulin resistance than those with higher socio-economic status, but the difference was not statistically significant. We also found that whites were as equally insulin-resistant as the rather heterogeneous group of non-whites, including 11 subjects of Mediterranean origin, 10 Africans, 15 Asians, and 4 others (data not shown), while it is known that prevalence rates of type 2 diabetes are substantially higher among blacks and Indians. The question arises whether selective response could account for (part of) the observed associations in our study sample. Statistical adjustment for a number of variables, including socio-economic status and race, in regression analyses hardly changed any of the coefficients between early growth and the parameters of insulin resistance. This makes bias introduced by selective response less likely, although it does not exclude the possibility completely. A possible relationship between birth weight SDS and the parameters of insulin resistance would be concealed if the low birth weight SDS subjects with a high risk of insulin resistance selectively declined to participate. Furthermore, the relationship between infancy weight gain and insulin level would be artificial if the slow weight gain subjects with a high risk of insulin resistance selectively refused to participate. This seems not very likely. Moreover, although non-response was associated with demographic factors linked to insulin resistance, in the entire birth cohort (N=1,012) there were no effects of race or socio-economic status on birth weight SDS or infancy weight gain.

Figure 1. Effects of birth weight SDS and adult absolute fat mass on log HOMA-IR at age 19 years.



LBW: lower birth weight SDS.
HBW: higher birth weight SDS.

HBW-lean: birth weight SDS ≥0 and absolute fat mass ≤median for sex (N=78).
LBW-lean: birth weight SDS <0 and absolute fat mass ≤median for sex (N=90).
HBW-obese: birth weight SDS ≥0 and absolute fat mass >median for sex (N=85).
LBW-obese: birth weight SDS <0 and absolute fat mass >median for sex (N=81).

Although the majority of full-term small-for-gestational-age (SGA) babies show catch-up growth after birth (34), infancy weight gain of our study population was slow, which is reflected by the low mean weight SDS at 3 months and 1 year. However, in contrast to full-term SGA babies, many very preterm infants suffer from life-threatening conditions after birth which require a shift in energy expenditure, enabling them to survive at the expense of somatic growth; e.g. by respiratory distress necessitating assisted ventilation, or infections.

To date, a number of studies have investigated the effect of infancy weight gain on later type 2 diabetes (3;11;35;36). In middle-aged subjects, low weight at birth and at age 1 year were associated with type 2 diabetes, but the rate of weight gain in the first year of life was unrelated to type 2 diabetes (35). However, in 1-year-old infants born SGA, catch-up growth in weight until age 1 years was associated with higher fasting insulin levels (3). Similarly, in children aged 8 years, rapid weight gain between birth and age 3 years was related to insulin resistance (36). Also in prematurely born subjects aged 13 to 16 years, effects of early postnatal weight gain on later insulin resistance have been reported. Boys and girls with rapid weight gain in the first 2 weeks postnatally had higher concentrations of proinsulin and 32-33 split proinsulin (11). Our findings in young adults born very preterm support these previous observations in paediatric populations.

We found that lower birth weight SDS strongly modified the effect of greater absolute and relative fat mass on insulin and C-peptide levels as well as HOMA-IR in young adulthood, which is consistent with previous studies in full-term 20-year-old subjects (5). We also found an interaction effect between lower birth weight SDS and greater adult height SDS on insulin level and HOMA-IR, but this probably reflects the effect of greater absolute fat mass (which is closely related to height). The interaction between lower birth weight SDS and greater adult relative fat mass (which is independent of height) on the parameters of insulin resistance suggests an effect of adult fat accumulation rather than of height.

Conclusions

In conclusion, our study in a relatively large cohort of men and women born very preterm showed that rapid infancy weight gain until 3 months predicted higher insulin levels at age 19 years, but the association was weak. Adult fat accumulation strongly predicted higher insulin and C-peptide levels as well as higher HOMA-IR at age 19 years. The effect of adult fat accumulation on these parameters of insulin resistance was dependent on its interaction with birth weight SDS.

A previous study in the same cohort showed that rapid infancy weight gain was a strong predictor of adult fat accumulation (16), whereas this study showed only a weak effect of rapid infancy weight gain until 3 months on insulin level (and no effect on C-peptide level and

HOMA-IR) in young adulthood. At present, it is unclear whether interventions aimed at discouraging infancy weight gain would improve adult body composition and reduce the chance of developing type 2 diabetes. However, recent evidence suggests that rapid infancy weight gain of very preterm infants is beneficial for several neurodevelopmental outcomes (37;38), making intervention (i.e., undernutrition) hard to justify.

Recently, it was found that the survivors of very preterm birth are already more insulin-resistant at the age of 7 years (7). In line with these observations, we found that HOMA-IR (which normally approximates to 1 in young non-obese persons, if glucose is measured in mmol/l and insulin in mU/l (17)) was relatively high in our study population. Also, we found that our subjects had already some centralization of fat distribution compared with population references. As there is strong tracking of obesity from childhood to young adulthood (39), we would like to call upon paediatricians and primary healthcare workers to be alert to fat accumulation during childhood in the follow-up of very preterm subjects, especially of those born SGA, and to intervene, even though it has not yet been tested whether weight reduction in very preterm subjects can reverse insulin resistance. The question of whether the survivors of very preterm birth, and especially those born SGA who subsequently become overweight, have a premature onset of type 2 diabetes remains very interesting and should be addressed.

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7 Preterm birth and blood pressure in adulthood:

high prevalence of hypertension but no effects of intrauterine or infancy growth

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Abstract

Objectives:

To determine whether intrauterine growth retardation (IUGR) is a predisposing factor for high blood pressure in 19-year-olds born (very) preterm.

Methods:

Prospective follow-up study in 19-year-olds born preterm in The Netherlands in 1983. Systolic, diastolic, and mean blood pressure values and plasma renin activity concentration were obtained in 422 young adults born with a gestational age <32 weeks. Blood pressure was also measured in 174 individuals who were born with a gestational age of ≥32 weeks and a birth weight of <1,500 g.

Results:

An increased prevalence of hypertension and probably also of prehypertensive stage was found. IUGR, birth weight, gestational age, and plasma renin activity were not associated with blood pressure. Current weight and BMI were the best predictors of systolic blood pressure at the age of 19 years.

Conclusions:

The prevalence of hypertension is high in individuals who were born preterm when compared with the general population. In the individuals who were born very preterm, no support to the hypothesis that low birth weight is associated with increased blood pressure at young adult age can be given.

Introduction

The suggested association between birth weight and adult diseases was studied in many epidemiological studies in the past decades (1;2). In these studies, an inverse relation has been described between birth weight and hypertension, dyslipidaemia, type 2 diabetes, and cardiovascular diseases in adult life. Individuals born after intrauterine growth retardation (IUGR) are thought to be at risk of developing high blood pressure compared with subjects with the same birth weight but no IUGR (3;4).

Besides low birth weight, 3 other early factors that are considered to be important risk factors for developing high blood pressure in adult life have been identified in individuals with IUGR. First, accelerated postnatal growth in weight and height is suggested to increase the risk of developing hypertension and type 2 diabetes in later life, especially in individuals with a low birth weight (5;6).

Second, it was postulated that altered angiotensin activity was an important factor underlying the "fetal origins of adult diseases" hypothesis (7;8). Also hypoxia, increased sympathetic nerve activity, and catecholamine production and proliferation of juxta-glomerular cells (and thus renin-producing cells) are suggested as factors in the pathogenesis.

Finally, preterms are probably at even greater risk for developing adult diseases compared with individuals who were born at term. A large Swedish study showed an inverse association between gestational age, ranging from 35 to 44 weeks, and systolic blood pressure (SBP) in 165,136 Swedish men (9). This correlation may be stronger in the lower range of gestation (gestational age 30 to 38 weeks), as demonstrated by Siewert-Delle et al (10). Very preterm infants who were born with a gestational age of <30 weeks were not included in this study. In contrast, other studies do not support these data. Singhal et al did not find an attributable risk to vascular disease at the age of 15 years in 216 preterm individuals (mean gestational age: 31 weeks) compared with individuals who were of the same age and born at term (11).

It is suggested that the underlying mechanism for prematurity's influencing blood pressure and cardiovascular risk is related to an impaired (fetal) organ development. Many organ systems, such as kidneys and pancreas, develop until the third trimester of normal pregnancy. Preterm birth requires an increased energy of the neonate to grow and survive. Organ development, such as nephrogenesis and ß-cell development in the pancreas, is not or only partly completed after preterm birth (12). Large studies that include the lowest ranges of gestation are needed to explore the role of prematurity and growth retardation with respect to the "fetal origins of adult disease" hypothesis.

Also, several maternal factors, such as maternal hypertension, smoking during pregnancy, and perinatal and postnatal factors, such as Apgar score and comorbidity after birth and drug use, are supposed to influence both neonatal and adult health. To our knowledge, no previous prospective studies were able to analyze these potential confounders in the relation to birth weight and blood pressure.

In this article, we describe the results of a large prospective follow-up study in which blood pressure was obtained in 19-year-olds who were born in 1983 with a gestational age of <32 weeks. Within this cohort, our objective was to determine whether IUGR is associated with increased blood pressure at age 19 years after very preterm birth and whether this is amplified as a result of accelerated growth postnatally and to determine whether IUGR is associated with alterations in renin activity at age 19 years after very preterm birth. In addition, the effect of potential maternal, perinatal, and postnatal confounders on blood pressure at young adult age were studied, as well as the relation between gestational age and blood pressure.

Methods

Population

Subjects were recruited from the Project On Preterm and Small-for-gestational-age infants (POPS) cohort. The POPS cohort comprises of 94% of all Dutch neonates (N=1,338) who were born alive in 1983 with a gestational age of <32 weeks (group 1) and/or a birth weight of <1,500 g (group 2) (13). All individuals who were alive at the age of 19 years (N=959) and not lost to follow-up until the age of 14 years (N=934) were invited to participate in a prospective follow-up study conducted from April 2002 until May 2003 in 10 outpatient clinics in The Netherlands. The ethics committees of all participating centers approved the study protocol.

Perinatal parameters (e.g., birth weight, gestational age, Apgar score, congenital anomalies) and obstetric parameters (e.g., maternal hypertension, medication during pregnancy, smoking during pregnancy) were known since birth. Follow-up data for height, weight, and BMI until the age of 10 years were also known in almost all subjects.

Table 1. Characteristics of participants and non-responders.

Characteristic	Participants			Non-responders
	All subjects	Group 1: <32 weeks	Group 2: ≥32 weeks and <1,500 g	
N	588	418	170	363
Males (%)	44.9	46.7	41.4	62.8
Birth weight (g)	1,303±302	1,314±338	1,274±177	1,328±251
Gestational age (weeks)	30.9±2.5	29.7±1.5	33.9±1.6	31.2±2.6
Age (yrs)	19.3±0.2	19.3±0.2	19.3±0.2	-
SBP (mmHg)	123±12	123±13	122±12	-
DBP (mmHg)	66±8	66±8	66±8	-
MAP (mmHg)	85±9	85±9	85±8	-

Values represent mean±SD or percent.

Birth weight and birth length were converted to SD score (SDS), using Swedish reference standards (14). Birth weight SDS was considered as a measure of IUGR. At follow-up visits at the ages of 3, 6, and 12 months, weight and length (measured in supine position), and at the ages of 2, 5, 10, and 19 years, data on weight and height (measured in standing position) were recorded. All of these parameters were converted to SDS, using Dutch reference standards (15).

The main statistical analyses included only participants of group 1 (gestational age <32 weeks). To study the relation between gestational age and blood pressure, also subjects of group 2 (gestational age ≥32 weeks and birth weight <1,500 g) were included to increase the range of gestation until 40 weeks. Prevalence rates of hypertension were also calculated in both groups.

Study protocol

Informed consent was obtained after oral and written information had been given. SBP and diastolic blood pressure (DBP) were obtained with an automatic blood pressure device (Dinamap, Critikon, Germany). Three measurements were performed at the non-dominant arm in supine position after 30 minutes of rest in the same position. The cuff-size was adjusted for arm length and circumference. Mean values were used in the statistical analysis. Mean arterial pressure (MAP) was calculated as: (SBP + 2 x DBP) / 3. Information about medical history and drug use was obtained by an interview.

Individuals were excluded from the analyses when anti-hypertensive medication was used, individuals were pregnant, or blood pressure was not measured according protocol. Weight and height were recorded to the nearest 0.1 kg and 0.1 cm, respectively, using calibrated scales. Participants were categorized into normal blood pressure (SBP <120 mmHg and DBP <80 mmHg), prehypertensive blood pressure (SBP 120-139 mmHg or DBP 80-89 mmHg), hypertension stage 1 (SBP 140-159 mmHg or DBP 90-99 mmHg) or hypertension stage 2 (SBP >160 mmHg or DBP >100 mmHg) according to the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure VII criteria (JNC VII) (16).

Laboratory analysis

A blood sample was obtained after blood pressure measurement, in which plasma renin activity (PRA) was measured by quantification of the generated angiotensin I with a radioimmunoassay (Incstar, Stillwater, MN, USA). The sensitivity was 0.05 µg/l/h, and the coefficient of variation ranged from 5.6 to 7.6% at different levels.

Statistical analysis

Unpaired t tests were performed to compare blood pressure means. Because birth weight is positively associated with adult weight and adult weight influences blood pressure (causal pathway) (17), we used a multivariate regression model to analyze the effect of birth weight

on blood pressure and the effect of growing more (or less) in weight than would be expected from a given birth weight. Therefore we first used linear regression to calculate the expected size (weight or height), on the basis of birth weight, and then subtracted the actual size. This "residual" was entered in the final linear regression model (18). Recently, we explained the algebraic concept of this regression model (19). The coefficient of birth weight SDS shows the effect of birth weight SDS on adult blood pressure, and the coefficient of the residual weight shows the effect of gaining more (or less) weight than expected on adult blood pressure. Likewise, multiple logistic regression analyses were applied to evaluate the effect of birth weight SDS and residual weight adjusted for gender on the prevalence of hypertension. The effect of gestational age and gender on blood pressure and the prevalence of hypertension was analyzed separately with linear and logistic regression models. Statistical significance was defined as a P value <0.05.

Table 2. Regression analyses of birth weight SDS and postnatal weight gain on blood pressure at age 19 years.

Outcome	Birth weight	Weight						
	SDS	3 mo	6 mo	1 yr	2 yrs	5 yrs	10 yrs	19 yrs
SBP	0.500	0.556	0.696	0.667	0.383	1.736[a]	2.212[a]	2.324[a]
	(-0.667 to 1.668)	(-0.375 to 1.487)	(-0.395 to 1.788)	(-0.439 to 1.773)	(-0.790 to 1.556)	(0.708 to 2.764)	(1.102 to 3.321)	(1.433 to 3.215)
DBP	0.281	0.155	0.024	-0.435	-0.519	0.013	0.395	0.165
	(-0.490 to 1.051)	(-0.461 to 0.770)	(-0.745 to 0.696)	(-1.167 to 0.296)	(-1.294 to 0.256)	(-0.667 to 0.703)	(-0.373 to 1.163)	(-0.441 to 0.771)
MAP	0.354	0.288	0.216	-0.068	-0.219	0.588	1.000[b]	0.885[b]
	(-0.472 to 1.180)	(-0.372 to 0.949)	(-0.557 to 0.988)	(-0.851 to 0.715)	(-1.051 to 0.641)	(-0.149 to 1.324)	(0.194 to 1.807)	(0.240 to 1.529)

Values represent ß (95% CI). All analyses were adjusted for sex.

[a] P <0.01.

[b] P <0.05.

Table 3. Regression analyses of birth length SDS and postnatal length/height gain on blood pressure at age 19 years.

Outcome	Birth length	Length/height						
	SDS	3 mo	6 mo	1 yr	2 yrs	5 yrs	10 yrs	19 yrs
SBP	0.877	0.636	0.359	0.686	0.267	0.313[a]	1.126	0.822
	(-0.283 to 2.037)	(-0.544 to 1.817)	(-0.889 to 1.606)	(-0.597 to 1.970)	(-1.027 to 1.580)	(0.149 to 0.478)	(-0.158 to 2.409)	(-0.548 to 2.192)
DBP	0.641	0.258	-0.126	0.030	0.100	0.153[b]	-0.182	-0.400
	(-0.121 to 1.403)	(-0.509 to 1.025)	(-0.923 to 0.672)	(-0.869 to 0.810)	(-0.951 to 0.751)	(0.044 to 0.263)	(-1.068 to 0.703)	(-1.017 to 0.501)
MAP	0.720	0.384	0.036	0.209	0.026	0.207[b]	0.254	0.007
	(-0.096 to 1.536)	(-0.445 to 1.214)	(-0.832 to 0.904)	(-0.696 to 1.114)	(-0.886 to 0.938)	(0.090 to 2.005)	(-0.679 to 1.186)	(-0.959 to 0.973)

Values represent ß (95% CI). All analyses were adjusted for sex.

[a] P <0.01.

[b] P <0.05.

Results

Of 934 eligible subjects, 596 participated in this study (63.8% response rate). Of the 338 non-responders, 59 were lost to follow-up, 53 were not able, 177 did not feel like, and 27 did not have time to participate. Thirteen subjects could not be included within the research period, and in 9 subjects the reason for non-response is unknown.

Five subjects mentioned that they had had increased blood pressure in the past, but none was treated for hypertension at the time of the study. Eight subjects were excluded from the data analysis: 4 because of use of anti-hypertensive medication for other reasons than hypertension (e.g., restless legs, nervousness), 2 because of protocol violation, 1 because of pregnancy, and 1 because of unreliable blood pressure measurement. Therefore, SBP and DBP data of 588 subjects were analyzed, 264 of whom were male and 324 of whom female (Table 1). The mean age was 19.29 (range: 18.63 to 20.18) years. A total of 418 subjects were born at a gestational age of <32 weeks (group 1); 170 were born with a gestational age of ≥32 weeks and with a birth weight <1,500 g (group 2). Gestational age and birth weight of the subjects in group 1 were 29.7±1.53 weeks and 1,314 ±338 g and in group 2 were 33.9±1.63 weeks and 1,274±177 g, respectively.

Of all subjects who were alive at 19 years, birth weight and gestational age did not differ between the responders and non-responders. Baseline characteristics of the non-responders are shown in Table 1. Compared with the subjects in the original cohort (including those who died and were lost to follow-up), the responders had a higher mean birth weight (101 g; 95% CI of mean difference: 67 to 134 g) and a longer duration of gestational age (1.2 week; 95% CI of mean difference: 0.9 to 1.5 weeks).

Data of blood pressure values are shown in Table 1. SBP, DBP, and MAP in participants in group 1 was 123±13 mmHg, 66±8 mmHg, and 85±9 mmHg, respectively. Participants in group 2 had SBP, DBP, and MAP of 122±12 mmHg, 66±8 mmHg, and 85±8 mmHg, respectively. SBP was higher in men (126±12) than in women (120±12). DBP was lower in men (64±8) than in women (68±8). Prenatal (maternal hypertension and maternal smoking during pregnancy) and perinatal and postnatal parameters (alterations on cardiotocographic measurement, Apgar score, neonatal use of corticosteroids, sepsis, and respiratory distress status) all were related to birth weight SDS but not to SBP and DBP values (data not shown). Therefore, no statistical adjustment for these parameters was required.

In a linear regression analysis of subjects born with a gestational age of <32 weeks, blood pressure was not associated with birth weight, birth weight SDS, birth length, and birth length SDS, all adjusted for gender. Regression coefficients are given in Tables 2 and 3. Increased postnatal weight gain and BMI after the age of 5 years both were predictors for SBP at the age of 19 years. The strength of this relation increased with age. Height at 5 years of age predicted SBP, DBP, and MAP at age 19 years. However, the actual effect on both SBP and DBP was very

small (0.3-mmHg increase in SBP per 1 SD more increase in height than expected at age 5). Current weight SDS and current BMI SDS were the strongest predictors for SBP (ß=2.3 mmHg per 1 residual weight SDS and 2.4 mmHg per 1 residual BMI SDS, respectively). Early postnatal weight gain (birth to 2 years) and increase in length were not related to blood pressure at the age of 19 years.

Blood pressure was also not related to gestational age, both when only participants in group 1 were included (ß for SBP: -0.251 mmHg per week increase in gestational age; 95% CI: -1.016 to 0.514) and when all subjects were included (ß for SBP: -0.149 mmHg per week increase in gestational age; 95% CI: -0.542 to 0.245) or when only subjects with a birth weight of <1,500 g were included (ß for SBP: -0.150 mmHg per week increase in gestational age; 95% CI: -0.553 to 0.254).

PRA was inversely related to blood pressure adjusted for gender. The ß for SBP was -0.02 (95% CI: -0.032 to -0.011) mmHg per 1 µg/l/h; the ß for DBP was -0.0133 (95% CI: - 0.037 to -0.005) mmHg per 1 µg/l/h; the ß for MAP was -0.024 (95% CI: -0.039 to -0.009) mmHg per 1 µg/l/h. Regression coefficients were not different between subjects with low and high birth weight SDS. PRA was not related with birth weight SDS or gestational age (Table 4 for mean values within birth weight SDS tertiles).

The prevalence of hypertension was 10.5% and of prehypertensive stage was 45.9% within group 1 and 8.8% and 37.6%, respectively, within group 2 (Table 5). The crude risk for hypertension was higher in men (odds ratio: 2.7; 95% CI: 1.4 to 5.3) compared with women. Birth weight SDS and gestational age both did not affect the risk for hypertension. Increased postnatal weight gain and BMI after 5 years were predictors for the risk of hypertension at the age of 19 years, but current weight affected the risk the most.

Discussion

This article describes the results of the first large scale prospective study on the suggested association between IUGR and blood pressure at the age of 19 years in individuals who were born with a gestational age of <32 weeks and/or birth weight of <1,500 g. Our main finding was that we could not show a relation between birth weight SDS, Birth length SDS, or gestational age and adult blood pressure. Adjustment for height, a common procedure in paediatric blood pressure interpretation, did not reveal a relation between birth weight SDS and blood pressure either. Also accelerated postnatal growth or weight gain during the first months in life did not influence blood pressure at the age of 19 years. Current weight and BMI were the best predictors for blood pressure at age 19 years.

A remarkable finding was that in our 19-year-old cohort (group 1), the mean SBP was high. In our cohort (mainly white), the SBP in men was 126 mmHg and in women was 120 mmHg. In

Table 4. Blood pressure, plasma renin activity, and BMI SDS within tertiles of birth weight SDS in participants of group 1.

Outcome	Tertiles of birth weight SDS			P for trend[a]
	Lowest	Middle	Highest	
SBP (mmHg)	122±13	123±12	124±13	0.73
DBP (mmHg)	66±8	66±8	66±8	0.91
MAP (mmHg)	85±9	85±8	85±9	0.95
PRA (µg/l/h)	2.5±1.3	2.2±1.0	2.3±1.5	0.33
BMI SDS	-0.3±1.3	-0.2±1.4	0 ±1.1	0.07

Values represent mean±SD.
[a] ANOVA.

comparison, the SBP of subjects of the same age (18 to 19 years) participating in the Bogalusa heart study was 115 mmHg in white men and 109 mmHg in white women (20). The Third National Health And Nutrition Examination Survey (NHANES III) reported a mean SBP in 17-year-olds of 117 mmHg in white boys and 107 mmHg in white girls (21). So, in both men and women, SBP was higher in our cohort. Other studies, such as NHANES and Framingham heart studies, reported mean blood pressure values in larger age categories (29 to 37 and 18 to 39 years), making comparison with our results difficult.

The prevalence of hypertension in our study was 10.5%. The overall prevalence of hypertension in subjects between 18 and 39 years of age was 7.2% in the NHANES III (22). Moreover, as it has been reported that the prevalence of hypertension increases by 1.3% with a 1-year increase of age (22), the prevalence of hypertension in the general population be-tween ages 18 and 39 would be higher than the prevalence in 19-year-olds. The prevalence of prehypertension in our cohort was 45.9%. Such individuals are suggested to have a 2-fold risk for progression toward hypertension in later life (16). Therefore, monitoring of blood pressure in these subjects is recommended. However, whether the prevalence of prehypertensive stage (45.9% in our cohort) was also high compared with the general population and/or with a random 19-year-old reference group is not known. Population-based reports on blood pressure prevalences according to the most recent criteria are needed. To compare our data with the Muscatine Study, we needed to categorize our data according the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure V criteria (23). Then, the prevalence of normal blood pressure (SBP <130 mmHg and DBP <85 mmHg) in our cohort was lower compared with the subjects in the Muscatine Study (62.4% versus 72%) and the prevalence of high blood pressure (SBP 130-139 mmHg or DBP 85-89) and hypertension stage I (SBP 140-159 mmHg or DBP 90-99) was higher (22.2% and 15.5% in our cohort versus 18% and 9% in the Muscatine, respectively) in both men and women (24). Again, the mean age of the subjects in our cohort was much lower: 19 years in our study versus 29 to 37 years in the Muscatine Study.

Several factors may have influenced our results. First, our protocol of blood pressure measurements to minimize variability deviated from other studies. We measured blood pressure 3 times in rest, in supine position, and at the non-dominant arm. Blood pressure values are suggested to be lower when measured in supine position, but when the arm is supported at the heart level (which is the case in supine position), no significant error is expected (25).

Second, our blood pressure values may have been influenced because of the use of automatic ossillometric device (Dinamap). Two studies that compared manometric and Dinamap blood pressure measurements reported an underestimation of the DBP values between 2.4 and 8.2 mmHg when this automatic device was used (26;27). However, inconsistency on the SBP values exists. Coppieters et al reported that SBP was systematically 3.6 mmHg lower in Dinamap measurements, but Pavlik et al reported a systematic overestimation of SBP between 1.0 and 6.7 mmHg when the Dinamap was used (26;27). When we adjusted for the systematic error as reported by Coppieters et al, the prevalence of hypertension increased to 18.9% and the prevalence of prehypertensive stage to 48.5%. When the Dinamap systematically overestimated the SBP with 6.7 mmHg and underestimated DBP with 2.4 mmHg (26;27), the mean blood pressure in our cohort decreased to 120±12 for SBP and 67±8 for DBP in men and 114±12 for SBP and 70±8 for DBP in females. The prevalence of hypertension within group 1 dropped to 4.3% and of prehypertensive stage to 31.8%. Still, mean SBP and DBP were higher compared with the subjects in the NHANES III and Bogalusa Heart Study (21;28).

Third, it is recommended that blood pressure be measured at least 2 times independently before mean SBP and DBP are calculated and a person is defined as hypertensive. Our 3 blood pressure measurements were performed on 1 day and are evidently not independent. However, the reference data that we used to compare prevalence rates and mean blood pressure values were also based on 1 initial screening and the mean of at least 3 measurements (21;22). Therefore, our data are comparable, showing both high prevalence and high mean blood pressure.

Table 5. Prevalence of hypertension using the blood pressure criteria according to the JNC VII.

Outcome	All subjects		Group 1: <32 weeks		Group 2: ≥32 weeks and <1,500 g	
	N	%	N	%	N	%
Normal blood pressure	273	46.4	182	43.5	91	53.5
Prehypertensive stage	256	43.5	192	45.9	64	37.6
Hypertension stage 1	55	9.4	42	10.0	13	7.6
Hypertension stage 2	4	0.7	2	0.5	2	1.2
Total	588	100.0	418	100.0	170	100.0

Our study indicates that individuals who were born preterm have elevated mean blood pressure values and that the prevalence of hypertension is increased at the age of 19 years. This is not related to the extent of IUGR (birth weight SDS), birth weight, or gestational age. In our cohort, the range of birth weight is 560 to 2,580 g. Most studies in which the relation between birth weight and adult blood pressure was found included subjects with a birth weight ranging between 2,000 and 5,000 g (29-31). This suggests that the relation among birth weight, prematurity, and blood pressure may be diminished in the lower birth weight ranges or gestational ages and is not a continuously linear but a curved dose-response relation. This would explain the increased mean blood pressure values, increased prevalence of hypertension, and the absence of the relation between birth weight and blood pressure in the participants of group 1 in our cohort. This trend has not been described in other studies that included preterm individuals who were born at gestational age of 30 weeks. Future studies may help to confirm our findings.

A few other reasons can be encountered for the absence of the association between IUGR and blood pressure. First, at 19 years of age, our cohort may have been too young to detect a relation. Possibly, the differences were not present at this age yet, or differences were too small to detect with our tools (which measures blood pressure 1 to 2 mmHg accurate). However, changes in blood pressure as a result of IUGR have been shown in other studies at even younger age (32). Law et al described that the effect of low birth weight on blood pressure may be obscured during adolescence (33;34). Follow-up of our subjects therefore is recommended.

Second, a selection bias could have been introduced because of a response of 64%. Of all subjects who were alive at 19 years of age, no differences in baseline characteristics were present. However, compared with the original cohort, the responders had a slightly higher birth weight and were born after a longer duration of gestation compared with the non-responders. So those with the suggested highest risk for increased blood pressure were less included in the study, possibly leading to negative results. Even if this bias were introduced and a relation between IUGR and blood pressure were concealed, our results concerning mean blood pressure values and prevalence rates are probably underestimated, and conclusions would not change much.

Finally, it is possible that the relation between birth weight and blood pressure does not exist at all and therefore was not found in our cohort. Indeed, authors have debated on contradictory results in several studies. Huxley et al stated that most studies that found a relation between birth weight and adult blood pressure included small numbers of subjects. With increasing study size, the relation diminished (35), suggesting a publication bias (36). Furthermore, most studies failed to account for possible appropriate adjustment for potential confounders, such as current weight (35). As birth weight is positively correlated with current weight and current weight with blood pressure, also in our cohort, current weight cannot be

designated as a potential confounder (causal pathway) (35;37;38). Therefore, we studied the effect of birth weight and current weight separately, using a multivariate regression model using "unexplained residuals" for current weight as adjusting variable (18). Using this model, no relation could be found.

Several authors suggested that other prenatal, perinatal, and postnatal parameters could influence the association of birth weight and blood pressure (35;38). In our study, we were able to show that maternal hypertension, smoking during pregnancy, neonatal corticosteroid use, presence of respiratory distress syndrome, and alterations on cardiotocographic measurement were associated with birth weight SDS. These factors were all not associated with blood pressure at 19 years. We therefore conclude that these parameters are not confounders in our study.

As expected, PRA was negatively correlated to blood pressure. Individuals with high levels of active renin will have lower blood pressure values. However, neither birth weight SDS nor gestational age was associated with PRA. These data are not in agreement with the findings of Martyn et al (8), who showed increased plasma concentrations of (in)active renin at adult age in subjects who were large at birth. Konje et al found that active renin concentrations in the umbilical vein in neonates after delivery was higher in individuals who were small-for-gestational-age (7). Both authors concluded that the renin-angiotensin system is altered in individuals with IUGR. Possibly, the relation between birth weight and PRA in our cohort is not found because the relation between birth weight and blood pressure is not present. Contribution to the pathophysiological mechanism of the renin-angiotensin system therefore cannot be given.

Conclusions

In conclusion, in this large cohort of young adults born prematurely, the mean SBP and the prevalence of hypertension were high at 19 years of age. No relation with IUGR was found. Therefore, in individuals who were born prematurely, no support for the "fetal origins of adult diseases" hypothesis can be given. Whether the relation between birth weight and blood pressure is a curved dose-response curve needs to be studied, using subjects in all birth weight ranges and gestational ages and sophisticated blood pressure tools.

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8 Antenatal glucocorticoid treatment for preterm birth is not associated with long-term metabolic risks

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Abstract

Objectives:

A single course of maternal glucocorticoid treatment is effective in reducing neonatal mortality after preterm birth. However, in animals, maternal glucocorticoid treatment is associated with lifelong hyperglycaemia and hypertension, and impaired nephrogenesis in offspring. Findings from studies in humans on this topic are highly contradictory due to a number of methodologic flaws and renal function after glucocorticoid exposure has never been assessed. Therefore, we assessed in a well-described population of preterm subjects whether antenatal glucocorticoid treatment for preterm birth is associated with long-term metabolic risks, including renal function, in adulthood.

Methods:

There were 365 19-year-olds born <32 gestational weeks from the Project On Preterm and Small-for-gestational-age infants cohort. Outcomes, i.e. body composition, insulin resistance, the serum lipid profile, blood pressure, and estimated renal function, were related to maternal betamethasone administered twice with a 24-h interval.

Results:

Neonatal survival in the betamethasone-exposed group was 82% compared to 70% among unexposed (log rank P=0.0016). We did not find any long-term adverse effects of antenatal betamethasone, with the exception of an effect on glomerular filtration rate (GFR) in women. In 19-year-old female survivors, GFR was lower after betamethasone: -7.4 (95% CI: -13.3 to -1.5) ml/min per 1.73 m^2.

Conclusions:

The reduction in neonatal mortality associated with a single course of maternal betamethasone is not accompanied by long-term metabolic risks in survivors of preterm birth. The only adverse effect found was lower GFR in women. Although this difference was not clinically relevant at 19 years, it might predict an increased risk of chronic renal failure in prematurely born women who were exposed antenatally to betamethasone.

Introduction

Neonatal survival after preterm birth has greatly improved in the past decades (1). One of the factors responsible is the widespread application of antenatal glucocorticoid treatment. This is effective in reducing the incidence of the respiratory distress syndrome after preterm birth (2). In animals, maternal glucocorticoid administration throughout, or during part of, gestation is associated with lifelong hyperglycaemia and hypertension, and impaired nephrogenesis in offspring (3-7). Recently, in the human, preterm birth has also been associated with subsequent insulin resistance and hypertension (8;9). However, due to a number of controversial findings by studies in survivors of preterm birth, it is still not known whether antenatal glucocorticoid exposure contributes to these associations.

One study in 177 adolescents aged 14 years found a higher blood pressure after antenatal exposure to betamethasone (10). However, possible effects of early glucocorticoid exposure on the onset and tempo of puberty invalidate a correct interpretation of these data, since pubertal stage has a large impact on blood pressure (reviewed in (11)). Conversely, another study in 81 individuals found a lower systolic blood pressure 20 years after betamethasone (12), whereas a larger study in 30-year-old offspring found that betamethasone was associated with higher insulin levels 30 minutes after an oral glucose load but not with blood pressure (13). However, in the latter, two-thirds of participants had received an inappropriately low dose of betamethasone, which is also reflected by the failure to demonstrate a reduction in neonatal mortality. Obviously, for those reasons, the long-term effects on offspring metabolic health after an appropriate dose of maternal glucocorticoids remain unclear. Moreover, to date, effects on renal function after antenatal glucocorticoid treatment have never been addressed.

Therefore, we studied in a large birth cohort of prematurely born men and women who were followed until 19 years of age the effect of a single treatment course of 24 mg maternal betamethasone on body composition, insulin resistance, the serum lipid profile, blood pressure, and estimated renal function.

Methods

Population

Subjects were drawn from the Project On Preterm and Small-for-gestational-age infants (POPS) cohort. The POPS study is a multicenter prospective follow-up study, comprising 94% of all liveborn very preterm (<32 gestational weeks) and/or very-low-birth-weight (<1,500 g) infants born in The Netherlands in 1983 (14), and has documented birth, growth, and various other characteristics from birth onwards (15). The POPS cohort originally comprised of 992 non-syndromic infants born before 32 weeks' gestation. In 3 children, no data on maternal betamethasone treatment was available.

Table 1. Perinatal characteristics of original birth cohort members and participants by antenatal betamethasone exposure.

Characteristic	Original birth cohort (N=989)			Participants at 19 yrs (N=365)		
	Exposed	Unexposed	P	Exposed	Unexposed	P
N	171	818	-	73	292	-
General						
Males (%)	105 (61%)	441 (54%)	0.08	43 (59%)	130 (45%)	0.03
Whites (%)	150 (88%)	692 (85%)	0.42	64 (88%)	255 (89%)	0.84
Parental socio-economic status (1-6)	2.9±1.8	2.6±1.9	0.05[a]	3.6±1.6	3.5±1.6	0.52[a]
Obstetric						
Maternal age (yrs)	27.5±5.3	26.4±6.4	0.04	27.1±5.7	27.0±5.6	0.85
Parity >0 (%)	82 (48%)	390 (48%)	0.98	36 (49%)	137 (47%)	0.73
Part of multiple pregnancy (%)	54 (32%)	203 (24%)	0.07	15 (21%)	72 (25%)	0.46
Hypertension during pregnancy (%)	18 (11%)	119 (15%)	0.17	6 (8%)	57 (20%)	0.02
Gestational diabetes (%)	7 (4%)	40 (5%)	0.65	2 (3%)	17 (6%)	0.39
Use of drugs and/or intoxication during pregnancy (%)[b]	87 (51%)	383 (47%)	0.33	39 (53%)	147 (50%)	0.64
Prolonged rupture of membranes (%)	45 (26%)	175 (21%)	0.16	20 (27%)	63 (22%)	0.29
Neonatal						
Gestational age (weeks)	29.4±1.7	29.1±2.0	0.04	29.6±1.4	29.8±1.5	0.22
Birth weight						
- g	1,293±330	1,235±353	0.05	1,346±319	1,330±339	0.72
- SD score	-0.05±0.88	-0.11±1.01	0.47	0.03+0.91	-0.13+1.05	0.19
Low Apgar score after 5 min (%)	32 (19%)	181 (25%)	0.13	6 (9%)	35 (13%)	0.29
Respiratory distress syndrome (%)	81 (47%)	482 (59%)	0.006	32 (44%)	146 (50%)	0.35
Intracranial haemorrhage (%)	42 (25%)	259 (32%)	0.07	18 (25%)	55 (19%)	0.27
Convulsions (%)	7 (4%)	59 (7%)	0.14	1 (1%)	8 (3%)	0.69
Necrotising enterocolitis (%)	6 (4%)	46 (6%)	0.24	2 (3%)	20 (7%)	0.27
Sepsis (%)	65 (38%)	283 (35%)	0.45	31 (43%)	95 (33%)	0.12
Postnatal glucocorticoid treatment (%)	9 (5%)	60 (7%)	0.33	6 (8%)	26 (9%)	0.85

Values represent N(%) or mean±SD. Continuous variables were compared with the unpaired t test.
Dichotomous variables were compared by the χ^2 test or Fisher's exact test.
[a] Mann-Whitney U test.
[b] Smoking, drinking alcohol, or using soft drugs, hard drugs or methadone during pregnancy.

In 1983, 12 mg betamethasone, administered twice with a 24-h interval, was the only therapy used to accelerate fetal lung maturation in the Netherlands (16). In the early 1980s, fear for possible long-term deleterious effects on the unborn child refrained many Dutch obstetricians from administering betamethasone to mothers with impending preterm delivery, while others routinely prescribed this therapy (17). Thus, in the POPS study, the allocation to betamethasone could be considered as a random process, reflecting the prescribing obstetrician's own preference, rather than a clinical decision based upon the prognosis of the unborn child.

At 19 years of age, all 669 subjects alive were invited to participate in the POPS-19 study, of whom 415 gave written informed consent (62% response rate). Response in the POPS-19 study is described in detail elsewhere (18). The data of subjects with endocrine disease (N=0)

or systemic glucocorticoid therapy (N=2), as well as of pregnant women (N=1), were excluded for this specific study. The data of not-fasted subjects (N=18) were not analyzed either. Furthermore, 29 subjects failed to provide blood, resulting in a total of 365 subjects included in the statistical analyses at 19 years of age. This study was approved by the medical ethical committees of all centers participating in the POPS-19 study.

Study protocol

Subjects were seen between 8.30 and 10.00 h at one of the outpatient clinics of the 10 participating centers after an overnight fast. Assessors were blinded to the perinatal characteristics of the subjects, including betamethasone exposure.

After 30 minutes in supine position, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured 3 times consecutively with an automatic blood pressure device (Dinamap, Critikon, Germany) at the non-dominant arm. The mean values of these measurements were used in the statistical analyses. The cuff size was adjusted to fit arm length and circumference. Mean arterial blood pressure (MAP) was calculated as: (SBP + 2 x DBP) / 3. Venous blood was subsequently drawn. Thereafter, weight was measured to the nearest 0.1 kg on a balance scale, and height to the nearest 0.1 cm with a fixed stadiometer. Waist circumference was measured at 0.1-cm accuracy, with a flexible tape measure.

Laboratory analysis

Serum samples were stored at -80 ^{o}C and thawed only once immediately before analysis. Serum glucose, total cholesterol, triglyceride, and creatinin concentrations were measured in a fully automated computerized laboratory system with a Hitachi 747 (Hitachi, Tokyo, Japan) chemistry analyzer. Low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol were measured with a turbidimetric assay on a Hitachi 911. Insulin was measured with a highly sensitive radioimmunoassay (Linco, St Charles, MO 63304, USA). The sensitivity of this assay is 0.1 mU/l, and the interassay coefficient of variation ranges from 4.7 to 12.2% at different levels.

Homeostatic model assessment for insulin resistance index (HOMA-IR) was calculated (19). Insulin level and HOMA-IR correlate strongly with Si assessed by the frequently-sampled intravenous glucose tolerance test in young persons (20;21). Estimated glomerular filtration rate (GFR) was calculated with the Cockroft-Gault equation, adjusted for body surface area (ml/min per 1.73 m^2) (22).

Statistical analysis

In the entire birth cohort as well as among the responders at age 19 years, perinatal characteristics between betamethasone exposed and unexposed groups were compared with the unpaired t test for continuous variables and by the χ^2 square test for dichotomous variables, as appropriate. When necessary, Fisher's exact test was used. Neonatal (i.e., 28-day)

mortality rates between exposed and unexposed groups were compared with the Kaplan-Meier method.

Continuous outcomes at age 19 years were compared with the unpaired t test. Multivariate linear regression was used to assess the effect of antenatal betamethasone exposure adjusted for the potential confounders race (white or non-white), parental socio-economic status (≤2 or >2), and obstetric characteristics (presence or absence for each variable separately listed in Table 1). Variables with skewed distributions (insulin, HOMA-IR, and triglycerides) were ^{10}log-transformed before statistical comparison.

Results

Perinatal data

The mothers of 171 of the 989 non-syndromic children born before 32 gestational weeks (17%) were treated with betamethasone prior to delivery (Table 1). The prescription of betamethasone was associated with a slightly older maternal age and tended to associate with socio-economic status and multiple pregnancies.

In the offspring, betamethasone was associated with a lower incidence of the respiratory distress syndrome. Sixty-nine children were treated with glucocorticoids postnatally, mostly because of difficulties to wean them from the ventilator, and 9 of them were also exposed in utero. Among participants, betamethasone exposure was negatively associated with female gender and hypertensive disease during pregnancy.

Table 2. Characteristics of participants at age 19 years by sex.

Characteristic	Men	Women	P
N	173	192	-
Height (cm)	179.4±7.8	166.4±7.2	<0.001
BMI (kg/m^2)	21.7±3.0	21.6±3.2	0.96
Waist circumference (cm)	80.1±8.7	76.7±8.6	<0.001
Glucose (mmol/l)	5.18±0.42	4.81±0.37	<0.001
Log Insulin (mU/l)	0.93±0.19	0.93±0.17	0.69
Log HOMA-IR	0.29±0.20	0.26±0.18	0.23
Total cholesterol (mmol/l)	3.92±0.81	4.45±0.84	<0.001
HDL cholesterol (mmol/l)	1.19±0.24	1.45±0.32	<0.001
LDL cholesterol (mmol/l)	2.34±0.72	2.62±0.76	<0.001
Log Triglycerides (mmol/l)	-0.11±0.21	-0.06±0.21	0.08
SBP (mmHg)	126.0±12.1	120.7±12.6	<0.001
DBP (mmHg)	63.8±7.7	68.2±8.5	<0.001
MAP (mmHg)	84.5±8.2	85.7±9.2	0.20
Creatinin (µmol/l)	87.6±8.9	77.1±8.4	<0.001
GFR (ml/min per 1.73 m^2)	110.0±13.9	103.1±15.4	<0.001

Values represent mean±SD. Variables were compared with the unpaired t test.

Table 3. Effect of antenatal betamethasone exposure on the metabolic profile at age 19 years.

3A. Men

Outcome	Antenatal betamethasone exposure		Mean difference (95% CI)	
	Exposed	Unexposed	Crude	Adjusted[a]
N	43	130	-	-
Height (cm)	179.0±7.7	179.5±7.8	-0.6 (-3.3 to 2.2)	-0.9 (-3.6 to 1.8)
BMI (kg/m^2)	21.5±2.5	21.7±3.2	-0.2 (-1.3 to 0.8)	-0.3 (-1.3 to 0.7)
Waist circumference (cm)	78.4±7.2	80.8±9.1	-2.4 (-5.4 to 0.7)	-2.8 (-5.8 to 0.1)
Glucose (mmol/l)	5.10±0.45	5.21±0.41	-0.12 (-0.26 to 0.03)	-0.10 (-0.25 to 0.04)
Log Insulin (mU/l)	0.88±0.20	0.94±0.18	-0.06 (-0.13 to 0)	-0.06 (-0.12 to 0.01)
Log HOMA-IR	0.23±0.22	0.31±0.20	-0.07 (-0.14 to 0)	-0.07 (-0.14 to 0.01)
Total cholesterol (mmol/l)	3.84±0.82	3.94±0.81	-0.09 (-0.38 to 0.18)	-0.06 (-0.34 to 0.23)
HDL cholesterol (mmol/l)	1.18±0.22	1.19±0.24	-0.01 (-0.09 to 0.07)	-0.01 (-0.09 to 0.08)
LDL cholesterol (mmol/l)	2.32±0.77	2.35±0.70	-0.02 (-0.28 to 0.23)	0.02 (-0.24 to 0.27)
Log Triglycerides (mmol/l)	-0.13±0.19	-0.09±0.21	-0.04 (-0.11 to 0.03)	-0.04 (-0.12 to 0.03)
SBP (mmHg)	123.2±12.0	126.9±12.1	-3.7 (-7.9 to 0.4)	-3.3 (-7.5 to 0.8)
DBP (mmHg)	61.3±7.8	64.6±7.5	-3.3 (-5.9 to -0.7)	-3.4 (-6.0 to -0.7)
MAP (mmHg)	81.9±8.1	85.4±8.1	-3.4 (-6.2 to -0.6)	-3.4 (-6.2 to -0.6)
Creatinin (µmol/l)	88.7±9.0	87.2±8.9	1.5 (-1.6 to 4.6)	1.4 (-1.8 to 4.5)
GFR (ml/min per 1.73 m^2)	107.7±11.8	110.8±14.5	-3.1 (-8.0 to 1.8)	-3.0 (-7.8 to 1.7)

Values represent mean±SD.

[a] Adjusted for race (white or non-white), socio-economic status (≤2 or >2), and obstetric characteristics (presence or absence).

3B. Women

Outcome	Antenatal betamethasone exposure		Mean difference (95% CI)	
	Exposed	Unexposed	Crude	Adjusted[a]
N	30	162	-	-
Height (cm)	166.7±8.1	166.4±7.1	0.3 (-2.5 to 3.2)	-0.2 (-3.1 to 2.7)
BMI (kg/m^2)	21.5±3.1	21.7±3.2	-0.1 (-1.4 to 1.2)	-0.1 (-1.4 to 1.1)
Waist circumference (cm)	75.9±9.3	76.9±8.5	-1.0 (-4.4 to 2.4)	-1.3 (-4.8 to 2.2)
Glucose (mmol/l)	4.78±0.34	4.82±0.38	-0.04 (-0.18 to 0.11)	-0.05 (-0.19 to 0.10)
Log Insulin (mU/l)	0.87±0.16	0.95±0.17	-0.08 (-0.15 to -0.01)	-0.08 (-0.15 to -0.01)
Log HOMA-IR	0.19±0.18	0.28±0.18	-0.08 (-0.15 to -0.01)	-0.08 (-0.16 to -0.01)
Total cholesterol (mmol/l)	4.38±0.74	4.46±0.86	-0.08 (-0.41 to 0.25)	-0.14 (-0.47 to 0.20)
HDL cholesterol (mmol/l)	1.43±0.24	1.45±0.33	-0.02 (-0.15 to 0.10)	-0.02 (-0.16 to 0.11)
LDL cholesterol (mmol/l)	2.52±0.73	2.64±0.76	-0.12 (-0.42 to 0.18)	-0.14 (-0.45 to 0.17)
Log Triglycerides (mmol/l)	-0.06±0.23	-0.06±0.21	0 (-0.08 to 0.08)	-0.01 (-0.09 to 0.07)
SBP (mmHg)	117.9±11.0	121.2±12.9	-3.4 (-8.3 to 1.6)	-3.3 (-8.4 to 1.8)
DBP (mmHg)	66.5±7.5	68.5±8.6	-2.1 (-5.4 to 1.2)	-1.9 (-5.3 to 1.4)
MAP (mmHg)	83.6±8.0	86.1±9.4	-2.5 (-6.1 to 1.1)	-2.4 (-6.1 to 1.3)
Creatinin (µmol/l)	80.9±8.1	76.4±8.3	4.5 (1.2 to 7.7)	4.7 (1.4 to 7.9)
GFR (ml/min per 1.73 m^2)	97.4±11.5	104.1±15.8	-6.7 (-12.8 to -0.6)	-7.4 (-13.3 to -1.5)

Values represent mean±SD.

[a] Adjusted for race (white or non-white), socio-economic status (≤2 or >2), and obstetric characteristics (presence or absence).

The betamethasone exposed and unexposed groups differed in neonatal survival, in favour of the exposed group (Figure 1; log rank P=0.0016). Cumulative survival was already significantly different at 24 hours after birth (P=0.009). This difference increased up to the age of 28 days. At that age, 82% of the betamethasone exposed children were still alive compared to 70% of the unexposed. The odds ratio for neonatal mortality after betamethasone exposure was 0.51 (95% CI: 0.33-0.77).

Data obtained at age 19 years

At 19 years of age, there were gender-specific differences between men and women. Men had a larger waist circumference and higher fasting glucose levels than women (Table 2). Moreover, SBP, creatinin levels, and GFR were higher in men. Women, in turn, had higher fasting cholesterol levels and DBP than men.

In general, there were no adverse effects in 19-year olds after antenatal betamethasone exposure, with only one exception (GFR in women, see below). Betamethasone exposed and unexposed persons did not differ for height, BMI, waist circumference, and cholesterol levels (Tables 3A and 3B). Men exposed to betamethasone had significantly lower DBP and MAP. These observations persisted after correction for race, socio-economic status, and obstetric characteristics. Exposed men had a tendency towards lower insulin levels and HOMA-IR, but these observations were not statistically significant. Exposed women had significantly lower insulin levels and HOMA-IR. Their creatinin levels were significantly higher and their GFR lower. All of these relations in women persisted after adjustment for race, socio-economic status, and obstetric characteristics. In both men and women, restriction of analyses to subjects born after non-hypertensive pregnancies did not alter the results (data not shown).

Discussion

The overall conclusion of our study is that the short-term benefits of a single treatment course of maternal betamethasone grossly outweigh the potential long-term metabolic risks in survivors of very preterm birth.

We found that women who were exposed antenatally to betamethasone had a lower GFR, although within normal limits, at 19 years of age. This finding is in line with studies in rats, showing a reduction in glomerular numbers and function after maternal glucocorticoid treatment (4;6), even after short treatment with corticosterone (23), the principal glucocorticoid in rats.

Contrary to expectation, antenatal betamethasone exposure was associated with lower fasting insulin levels and HOMA-IR, and, in men, it was also associated with lower DBP and MAP. Therefore, with the exception of lower GFR in women, we did not find any long-term adverse effects of antenatal exposure to betamethasone.

There are several potential limitations to the design of our study. First, non-randomized allocation of a treatment, such as in our study, carries the risk of selection bias. It is unknown whether the differences in characteristics known at the time of betamethasone prescription were due to random fluctuation or created by the obstetricians involved. However, statistical adjustment for these characteristics did not change our results. Moreover, it was recently explained by Vandenbroucke that the side effects of a therapy can be studied with an observational study as reliably as with a randomized trial, provided that these cannot be predicted from any clinical data at the time of prescription (24). For our study, it seems fair to assume that the lack of knowledge in 1983 about potential long-term metabolic risks of antenatal glucocorticoid treatment has precluded obstetricians from guiding their decisions by relevant prognostic data (e.g., positive family history for diabetes mellitus, chronic renal failure or cardiovascular disease).

Second, as we had incomplete follow-up data, it may be argued that the observed associations could be biased by non-response. Possible relations between betamethasone exposure and outcome variables would be concealed if either the betamethasone exposed or the unexposed subjects with an adverse metabolic profile selectively declined to participate. This seems not very likely.

Third, our results pertain to individuals born at a gestational age <32 weeks. Subjects exposed to betamethasone before 32 weeks and born later were not part of the sample.

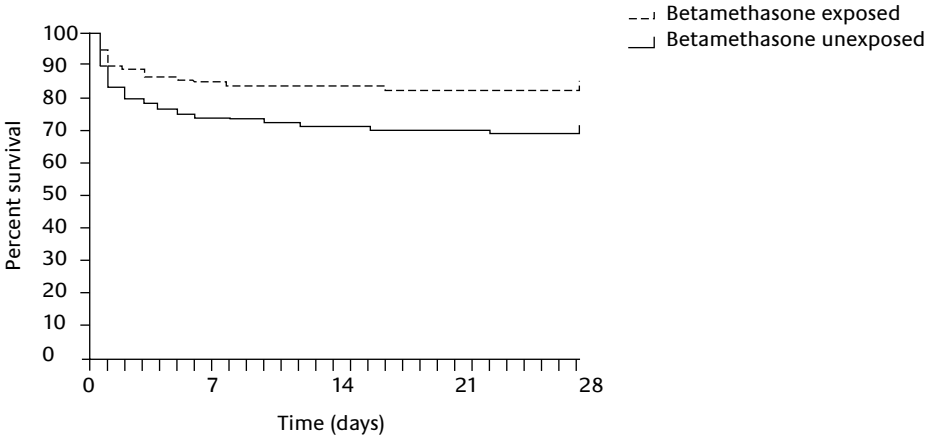
The difference in neonatal survival between the betamethasone exposed and unexposed groups was associated with a lower incidence of the respiratory distress syndrome in the exposed group, in line with the data of a meta-analysis of randomized trials (1). Betamethasone readily crosses the placenta, resulting in high cord vein glucocorticoid bioactivity that returns to the reference level within1 to 2 days after the last steroid dose (25). In preterm newborns, the effects of antenatal glucocorticoids are likely to be more pleiotropic, at least shortly after exposure, than merely reflected in a lower incidence of the respiratory distress syndrome. A recent study showed that preterm newborns who were exposed antenatally to betamethasone, had a transient elevation of insulin and C-peptide levels, and HOMA-IR in cord blood (26), which is indicative for insulin resistance. Furthermore, another study showed that treatment was associated with less need for blood pressure support during the first 48 hours after birth (27). In this regard, some comments should be made on the paradoxical influences of antenatal betamethasone exposure on adult blood pressure and markers of insulin resistance found in our study. In preterm newborns, hypotension is a strong predictor of severe intracranial haemorrhage, ischemic cerebral lesions, or death within 48 hours (28), and, in survivors, of poorer neurological outcome at term (29). There is lack of scientifically sound studies which address the prognostic value of neonatal hypoglycaemia in preterm newborns (30), with the exception of one study, showing that it is associated with adverse neurodevelopment at 18 months of age (31). Such observations stress the importance of adequate blood pressure and

glucose availability after preterm birth. Therefore, a possible explanation for the paradoxical associations in our study is that the neonatal survival of 'betamethasone depleted' newborns was more dependent on intrinsic factors associated with blood pressure regulation and glucose/insulin homeostasis, predisposing to later hypertension and insulin resistance.

Conclusions

We conclude that a single treatment course of maternal betamethasone does not adversely influence long-term metabolic health in very preterm offspring. The only adverse effect found was lower GFR in women. Although this difference was not clinically relevant at 19 years of age, it could predict an increased risk of chronic renal failure in prematurely born women who were exposed antenatally to betamethasone. Long-term follow-up of this study population is necessary to address this possibility. Persistent effects of multiple courses of maternal glucocorticoids in their children need to be explored by future studies.

Figure 1. Neonatal survival after very preterm birth in betamethasone exposed (N=171) and unexposed groups (N=818).



Dashed line represents the betamethasone exposed group. During the neonatal period, 30 events had occurred in the exposed group compared to 242 in the unexposed group. The odds ratio for neonatal mortality after betamethasone exposure was 0.51 (95% CI: 0.33 to 0.77).

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9 The 23K variant of the R23K polymorphism in the glucocorticoid receptor gene protects against postnatal growth failure and insulin resistance after preterm birth

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Abstract

Objectives:

Preterm birth is associated with postnatal growth failure, abdominal fat accumulation, insulin resistance, and hypertension, resembling increased glucocorticoid bioactivity. We tested the effects of the R23K and N363S polymorphisms in the glucocorticoid receptor gene, associated with decreased and increased sensitivity to cortisol, respectively, on linear growth and the adult metabolic profile in a cohort of men and women born <32 gestational weeks and followed prospectively from birth until 19 years of age.

Methods:

This was a birth cohort study that included 249 19-year-old survivors born at a gestational age <32 weeks from the Dutch Project On Preterm and Small-for-gestational-age infants cohort. Outcomes were linear growth and adult body composition, and fasting cortisol, glucose, insulin, and cholesterol concentrations, and blood pressure.

Results:

The 23K variant (N=24) was associated with lower fasting insulin levels (mean difference after log-transformation: -0.09 (95% CI: -0.16 to -0.01) mU/l) and a lower homeostatic model assessment for insulin resistance index (mean difference after log-transformation: -0.09 (95% CI: -0.16 to -0.01)), as well as with a taller stature departing from the age of 1 year onwards. 23K carriers showed complete catch-up growth between the ages of 3 months and 1 year and attained height was similar to the population reference mean, whereas stature in non-carriers was on average 0.5 SD below this mean. In contrast, the N363S polymorphism was not associated with any of the outcomes.

Conclusions:

Carriers of the 23K variant are, at least in part, protected against postnatal growth failure and insulin resistance after preterm birth.

Introduction

Functional changes in the gene encoding the glucocorticoid receptor (GR) play an important role in glucocorticoid bioactivity. To date, several functional polymorphisms in the GR gene have been identified, including R23K (ER22/23EK) and N363S. The 23K variant has been associated with decreased sensitivity to glucocorticoids and a beneficial metabolic health (1). Elderly persons with this variant had lower levels of fasting insulin, and of total and low-density lipoprotein (LDL) cholesterol (1). Thirty-six-year-old men with this variant had a taller stature, more lean body mass, and a greater muscle strength, while their female contemporaries had a tendency towards a smaller waist circumference (2). Unfavourable effects were found with the 363S variant, which has been associated with increased sensitivity to glucocorticoids (3). Subjects carrying this variant were predisposed to obesity and coronary artery disease (3-6).

A number of recent studies have elucidated the long-term metabolic consequences of preterm birth. These consequences include an increased risk of short stature (7;8), abdominal fat accumulation (9), insulin resistance (10-12), and hypertension (12-14). The clustering of postnatal growth failure and metabolic risk factors in prematurely born persons resembles effects of increased glucocorticoid bioactivity. Indeed, in a small sample of young adults, it was found that basal cortisol levels were higher after preterm birth (15). It is unknown whether common variants in the GR gene could explain variations in the endocrine-metabolic state of adults born prematurely.

Therefore, we tested the effects of the R23K and N363S polymorphisms on linear growth, body composition, insulin resistance, the serum lipid profile, and blood pressure in a cohort of 19-year-old men and women who were born very preterm (i.e., <32 gestational weeks).

Methods

Population

The Project On Preterm and Small-for-gestational-age infants (POPS) study is a nationwide multicenter prospective follow-up study, comprising 94% of all liveborn very preterm (<32 weeks' gestation) and/or very-low-birth-weight (<1,500 g) infants born in The Netherlands in 1983 and has documented birth, growth, and a number of other characteristics from birth onwards (16;17). At follow-up visits at the ages of 3 and 6 months, and 1 year and 2 years post-term, and at the chronological age of 5 years, length/height was measured. Length until the age of 2 years was measured to the nearest 1 cm in supine position, fully extended with the heels in contact with a baseboard. Standing height at the age of 5 years was measured to 1-mm accuracy. At 19 years of age, all 637 alive subjects born with a gestational age <32 weeks who were free from congenital skeletal deformations, Down's syndrome, chromosomal

abnormalities, multiple congenital deformations or inborn errors of metabolism, and who were not born to mothers with gestational diabetes, were approached by mail to participate in the POPS-19 study. Of these subjects, 395 consented to participate (62% response rate). Subjects with diabetes mellitus, or on thyroid hormone or systemic corticosteroids, as well as non-white subjects and pregnant women, were excluded for this specific study. The data of not-fasted subjects were not analyzed either. The approval of the medical ethical committees of all participating centers was obtained for the POPS-19 study.

Study protocol

Subjects who gave written informed consent to participate were seen after an overnight fast between 8.30 and 10.00 h at one of the outpatient clinics of the 10 participating centers. Assessors were blinded with respect to the perinatal characteristics of the subjects.

After 30 minutes in supine position, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured 3 times consecutively with an automatic blood pressure device (Dinamap, Critikon, Germany) at the non-dominant arm. The mean values of these measurements were used in the statistical analyses. The cuff size was adjusted to fit arm length and

Table 1. Perinatal characteristics of participants by GR genotype.

Characteristic	R23/23K	N363/363S	Non-carriers	P	
				23K vs. non-carriers	363S vs. non-carriers
N	24	15	210	-	-
Males (%)	12 (50.0%)	6 (40.0%)	102 (48.6%)	0.89	0.52
Obstetric					
Maternal age (yrs)	28.1±4.5	27.5±3.3	27.2±5.4	0.44	0.82
Parity >0 (%)	12 (50.0%)	6 (40.0%)	100 (47.6%)	0.83	0.57
Part of multiple pregnancy (%)	2 (8.3%)	2 (13.3%)	54 (25.7%)	0.06	0.37
Hypertension during pregnancy (%)	8 (33.3%)	1 (6.7%)	35 (16.7%)	0.06	0.48
Drugs and alcohol intoxication (%)	9 (37.5%)	7 (46.7%)	113 (53.8%)	0.13	0.59
Prolonged rupture of membranes (%)	2 (8.3%)	4 (26.7%)	51 (24.3%)	0.08	0.76
Maternal glucocorticoid treatment (%)	7 (29.2%)	4 (26.7%)	42 (20.0%)	0.30	0.52
Body proportions at birth					
Gestational age (weeks)	30.2±1.4	30.0±1.5	29.9±1.5	0.34	0.73
Birth weight					
- g	1,321±268	1,449±411	1,335±327	0.83	0.20
- SDS	-0.33±0.93	0.19±0.91	-0.13±1.03	0.37	0.24
Postnatal clinical course					
Respiratory distress syndrome (%)	10 (41.7%)	7 (46.7%)	102 (48.6%)	0.52	0.89
Intracranial hemorrhage (%)	5 (20.8%)	2 (13.3%)	36 (17.1%)	0.58	1.00
Sepsis (%)	12 (50.0%)	4 (26.7%)	70 (33.5%)	0.11	0.59
Postnatal glucocorticoid treatment (%)	0	4 (26.7%)	14 (6.7%)	0.37	0.02

Values represent N(%) or mean±SD. Continuous variables were compared with the unpaired t test.
Dichotomous variables were compared by the χ^2 test or Fisher's exact test.

circumference. Venous blood was subsequently obtained in supine position. Thereafter, anthropometry was performed, for which assessors had received extensive training prior to the study, and re-training during the entire study period at 2-months' intervals. Subjects were measured barefoot while wearing underclothing only. Weight was measured to the nearest 0.1 kg on a balance scale, and height to the nearest 0.1 cm with a fixed stadiometer. Waist and hip circumferences were measured to 0.1-cm accuracy using standard methods (18). Four skinfold thickness measurements were taken in duplicate with a calibrated skinfold caliper on the left side of the body: at triceps, biceps, subscapular, and iliacal regions. From these measurements, fat mass was calculated using the equations of Durnin and Ramahan (19). A more detailed description of skinfold thickness measurements obtained in the POPS-19 study has been published elsewhere (9).

Table 2. Effect of the R23K and N363S polymorphisms on length/height SDS.

Follow-up visit	R23/23K		N363/363S		Non-carriers		Mean difference (95% CI)	
	Men	Women	Men	Women	Men	Women	23K vs. non-carriers	363S vs. non-carriers
N	12	12	6	9	102	108	-	-
Birth	-0.45±1.55	-0.12±0.71	-0.46±0.99	0.02±0.81	-0.10±1.14	-0.05±1.39	-0.21 (-0.86 to 0.44)	-0.09 (-0.89 to 0.71)
3 mo	-0.74±0.92	-1.21±0.94	-1.70±1.59	0.05±1.46	-0.99±1.41	-0.77±1.33	-0.09 (-0.69 to 0.50)	0.23 (-0.51 to 0.97)[a]
6 mo	-0.01±1.16	-0.57±0.97	-1.46±0.98	-0.48±0.73	-0.66±1.13	-0.83±1.26	0.44 (-0.12 to 0.99)	-0.15 (-0.80 to 0.50)
1 yr	0.69±1.10	-0.19±0.72	-0.74±0.76	-0.53±0.84	-0.51±1.12	-0.76±1.22	0.84 (0.30 to 1.38)	0.02 (-0.59 to 0.63)
2 yrs	0.49±0.81	-0.11±0.68	-0.45±0.62	-0.25±0.91	-0.60±1.22	-0.44±1.32	0.68 (0.29 to 1.07)	0.19 (-0.47 to 0.85)
5 yrs	0.51±0.81	-0.19±0.62	-0.65±0.53	-0.41±0.91	-0.62±0.97	-0.39±1.00	0.66 (0.25 to 1.08)[a]	0 (-0.52 to 0.51)
19 yrs	0.14±0.64	-0.31±0.60	-0.66±0.44	-0.11±0.97	-0.47±1.03	-0.48±1.11	0.39 (0.08 to 0.70)	0.15 (-0.41 to 0.70)

Values represent mean±SD.

[a] P <0.05 for gender-genotype interaction.

Laboratory analysis

Blood samples were stored at -80 °C and thawed only once immediately before analysis. Glucose and total cholesterol were measured in a fully automated computerized laboratory system with a Hitachi 747 (Hitachi, Tokyo, Japan) chemistry analyzer. High-density lipoprotein (HDL) and LDL cholesterol were measured with a turbidimetric assay on a Hitachi 911. Cortisol was measured with a fluorescence polarization immunoassay on an Abbott TDx (Abbott Laboratories, Abbott Park, IL 60064, USA). The sensitivity of this assay is 20 nmol/l and the interassay coefficient of variation ranges from 3.1 to 6.4% at different levels. Insulin and C-peptide were measured with highly sensitive radioimmunoassays (Linco, St Charles, MO 63304, USA). The detection levels of these assays are 0.1 mU/l and 0.03 mmol/l, respectively, and the interassay coefficients of variation range from 4.7-12.2% and 3.2-9.3% at different levels, respectively. Homeostatic model assessment for insulin resistance index (HOMA-IR) was calculated (20). Insulin and C-peptide levels, and HOMA-IR were used as parameters of insulin resistance.

Table 3. Effect of the R23K and N363S polymorphisms on the metabolic profile at age 19 years.

Outcome	R23/23K		N363/363S		Non-carriers		Mean diference (95% CI)	
	Men	Women	Men	Women	Men	Women	23K vs. non-carriers	363S vs. non-carriers
N	12	12	6	9	102	108	-	-
BMI SDS	-0.44±1.04	0.05±0.55	0.07±1.26	0.15±1.01	-0.20±1.05	-0.14±1.13	-0.03 (-0.48 to 0.43)	0.29 (-0.29 to 0.86)
Waist SDS	0.20±1.05	0.71±0.50	0.57±1.14	0.94±0.97	0.20±1.01	0.78±0.92	-0.04 (-0.46 to 0.38)	0.30 (-0.23 to 0.83)
Waist-to-hip ratio SDS	0.53±0.90	0.57±0.41	0.66±0.67	0.85±0.85	0.73±0.95	0.94±0.92	-0.30 (-0.61 to 0.02)	-0.08 (-0.56 to 0.42)
Absolute fat mass (kg)	11.1±5.3	19.2±3.1	11.8±6.8	20.5±6.7	11.5±5.0	18.4±5.8	-0.1 (-2.9 to 2.7)	2.4 (-1.2 to 5.9)
Percentage body fat (%)	15.1±4.7	31.2±3.7	15.7±6.1	31.2±5.3	16.2±5.0	29.9±6.4	-0.4 (-4.3 to 3.5)	2.5 (-2.4 to 7.4)
Log Insulin (mU/l)	0.82±0.13	0.91±0.16	0.85±0.17	0.91±0.19	0.95±0.19	0.95±0.17	-0.09 (-0.16 to -0.01)	-0.06 (-0.16 to 0.03)
C-peptide (mmol/l)	0.59±0.15	0.66±0.19	0.48±0.13	0.68±0.16	0.68±0.24	0.69±0.21	-0.06 (-0.15 to 0.04)	-0.08 (-0.20 to 0.04)
Log HOMA-IR	0.17±0.16	0.24±0.16	0.23±0.19	0.23±0.19	0.30±0.20	0.28±0.18	-0.09 (-0.16 to -0.01)	-0.06 (-0.16 to 0.04)
Total cholesterol (mmol/l)	3.61±0.58	4.85±0.58	4.13±0.50	4.59±0.96	3.96±0.84	4.41±0.87	0.04 (-0.33 to 0.42)[a]	0.22 (-0.25 to 0.68)
HDL (mmol/l)	1.27±0.25	1.37±0.22	1.40±0.37	1.54±0.35	1.20±0.24	1.44±0.35	-0.01 (-0.14 to 0.13)	0.16 (-0.01 to 0.33)
LDL (mmol/l)	2.06±0.42	3.02±0.61	2.34±0.67	2.68±0.84	2.36±0.74	2.59±0.76	0.06 (-0.26 to 0.38)[a]	0.05 (-0.36 to 0.46)
SBP (mmHg)	126±13	118±12	123±5	122±12	127±12	121±12	-2 (-7 to 3)	-1 (-8 to 5)
DBP (mmHg)	63±9	66±9	65±6	69±9	65±8	68±8	-2 (-6 to 1)	1 (-3 to 5)
Log Cortisol (nmol/l)	2.62±0.12	2.92±0.12	2.60±0.12	2.88±0.20	2.59±0.14	2.79±0.24	0.08 (-0.02 to 0.17)	0.07 (-0.05 to 0.19)

Values represent mean±SD.

[a] P <0.05 for gender-genotype interaction <0.05.

Fasting insulin levels and HOMA-IR correlate strongly with Si assessed by the frequently-sampled intravenous glucose tolerance test in young persons (21;22).

For both R23K (rs6190) and N363S (rs6195) polymorphisms, polymerase chain reactions were performed using 2.5 ng of genomic DNA and standard reagents. Polymorphisms were subsequently genotyped by mass spectrometry (homogeneous mass array system, Sequenom Inc, San Diego, CA, USA), using standard conditions. Genotypes were analyzed by using Genotyper 3.0 software (Sequenom Inc). We identified 24 subjects (9.6%) who were heterozygous for the 23K variant (12 men and 12 women), and 15 (6.0%) who were heterozygous for the 363S variant (6 men and 9 women). None of the subjects was carrier of both variants. The corresponding allele frequencies of 4.8% and 3.0%, respectively, were reasonably well in range with

the allele frequencies observed in healthy Dutch populations, ranging from 3 to 4.5% for the 23K variant, and from 3 to 5% for the 363S variant (1-3;23;24). For both polymorphisms, the genotype distribution was in agreement with the distribution predicted by the Hardy-Weinberg equilibrium (P=0.42 for the R23K polymorphism, and P=0.62 for the N363S polymorphism).

Statistical analysis

Auxological data at birth and on subsequent occasions were converted to SD score (SDS), to correct for (gestational) age and sex, using Swedish references for preterm infants (25) and recently collected Dutch references (18;26;27), respectively. Comparisons were made between minor allele carriers and non-carriers, using the unpaired t test. Outcomes with skewed distributions (cortisol, insulin, and HOMA-IR) were ^{10}log-transformed prior to statistical comparison. Analyses were repeated with adjustment for perinatal factors (obstetric characteristics, gestational age, and postnatal clinical course) using linear regression analysis. Modification by gender of the effect of genotype on outcomes was tested by first including the variables genotype (in which minor allele carrier=1 and non-carrier=0) and gender (in which male=1 and female=0) in a linear regression analysis followed by the inclusion of their product.

Results

Table 1 lists the perinatal characteristics of the 249 participants, showing that, apart from an unequal distribution in the numbers who had been treated with glucocorticoids as neonates, there were no statistically significant differences between the GR genotypes.

Table 2 summarizes the growth patterns of the groups up to adult height. 23K carriers and non-carriers showed a similar degree of catch-down growth between birth and the age of 3 months. Between the ages of 3 months and 1 year, 23K carriers showed more rapid catch-up growth than non-carriers. Stature at 1 year and beyond was greater than or similar to the population reference mean in carriers of the 23K variant, whereas in non-carriers it was on average 0.5 SD below this mean. Correction for perinatal factors only slightly reduced the strength of these associations (data not shown). Figures 1A-C show that the difference in linear growth between 23K carriers and non-carriers was more pronounced in men, though the direction of association was similar for women. Despite these sex-specific observations, the test for interaction showed that the association between the R23K polymorphism and stature was dependent on gender only at 5 years of age. Linear growth did not differ between 363S carriers and non-carriers.

Table 3 shows the adult metabolic profile for the GR genotypes. 23K carriers had lower fasting insulin levels and a lower HOMA-IR than non-carriers. These differences became somewhat larger after correction for perinatal factors (data not shown). In addition, 23K carriers had a

lower waist-to-hip ratio, but this observation did not reach statistical significance. Interaction between the R23K polymorphism and gender on total and LDL cholesterol levels was observed, which was explained by opposite influences in men and women of the 23K variant on cholesterol levels. The adult metabolic profile of 363S carriers did not differ from that of non-carriers.

Discussion

In this prospective study in subjects who were born very preterm (i.e., <32 gestational weeks) and followed until 19 years of age, we found that the 23K variant in the GR gene was associated with lower fasting insulin levels and a lower HOMA-IR, as well as with a taller stature departing from the age of 1 year. It was also associated with a smaller waist-to-hip circumference, though this observation was not statistically significant. Carriers of the 23K variant showed complete catch-up growth between the ages of 3 months and 1 year and attained height was similar to the population reference mean. The N363S polymorphism was not associated with any of these outcomes.

In our study, we found that mean adult stature in carriers of the 23K variant was similar to the population reference mean, whereas mean height in the non-carriers was approximately 0.5 SD below this mean. Previous studies in healthy men of different ages found that 23K carriers were on average 4 cm taller than non-carriers (2;24). This difference in final height was for an important part attributed to the pubertal growth spurt (2). In contrast, we found in our specific population of very preterm subjects that the growth pattern differed significantly between carriers and non-carriers already by the age of 1 year. However, the difference (in SDS) between 23K carriers and non-carriers did not further increase after puberty.

In addition, in this specific cohort, we found that the 23K variant was associated with lower fasting insulin levels and a lower HOMA-IR at only 19 years of age, in line with findings from others in elderly people (1). Observations in other cohorts of effects of the R23K polymorphism on body composition and the serum lipid profile (1;2) were not confirmed by our data. Furthermore, we found no statistically significant relations with blood pressure.

Experiments in rats have shown that non-handling during early postnatal development permanently increases hypothalamus-pituitary-adrenal (HPA) axis activity (28). Similar effects in offspring were observed with naturally low-grooming mothers (29). Furthermore, it has been indicated that the extent of grooming in rat mothers specifically alters the methylation at the GR gene promoter in the hippocampus, thereby explaining how the effect of maternal care might persist into adulthood (30). During their neonatal course, preterm newborns are to a large extent devoid of maternal care and, instead, are subject to many stressful and sometimes even critical events, including for example respiratory distress, intubation and mechanical ventilation, and frequent blood sampling. Therefore, it could be possible that adverse postnatal

circumstances in humans may also result in life-long activation of the HPA axis. This is supported by data from a small study in young adults, showing that basal cortisol levels are elevated after preterm birth (15). The current findings suggest that the 23K variant protects, at least in part, against postnatal growth failure and insulin resistance after preterm birth. We speculate that an extreme stressful event such as preterm birth may induce hypermethylation of the GR promoter, leading to less GR expression in central feedback regions and, hence, enhanced stress responsiveness. GR expression in carriers of the 23K variant may be less vulnerable to alterations in DNA methylation. Indeed, single-nucleotide polymorphisms have been shown to be associated to methylation of neighbouring CpG sites (31).

The functionality of the studied variants has been elucidated previously. The 23K variant has been associated with higher circulating cortisol levels after overnight dexamethasone suppression (1), whereas the 363S variant has been associated with lower post-dexa cortisol (3). These findings were subsequently confirmed by transfection studies, showing decreased and increased gene expression in response to GR binding, respectively (32).

Because our participants were genotyped at 19 years of age, a survivor effect of GR variation could not be excluded, considering the high neonatal mortality rate in the original cohort (16;17). However, an argument against selective survival of a particular genotype is that the observed genotype frequencies of the studied polymorphisms did not deviate much from the genotype frequencies in the normal Dutch population, implying that gross selective survival of a particular genotype is not very likely to have occurred in our population.

Although sex-specificity of the effects of the R23K polymorphism on linear growth and body composition have been reported by 1 study (2) and was attributed to a different regulation of HPA axis activity by androgens and estrogens, we did not find much evidence for sexually dimorphic effects of the R23K polymorphism, except for height at 5 years of age and adult cholesterol levels. Clearly, any sex-specific observation must be balanced against the small numbers of subjects carrying the 23K variant (12 men and 12 women).

Conclusions

In conclusion, we found in 19-year-old survivors of very preterm birth that the 23K variant was associated with lower fasting insulin levels and a lower HOMA-IR as well as with a taller stature departing from the age of 1 year. Carriers of the 23K variant showed complete catch-up growth between the ages of 3 months and 1 year and attained height was similar to the population reference mean. Therefore, carriers of the 23K variant are, at least in part, protected against postnatal growth failure and insulin resistance after preterm birth.

Figures 1A-C. Effect of the R23K polymorphism on linear growth.

Values represent mean±95% CI.

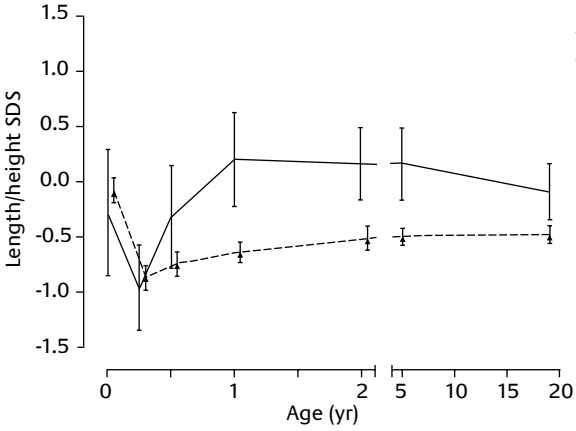

Figure A. Entire population

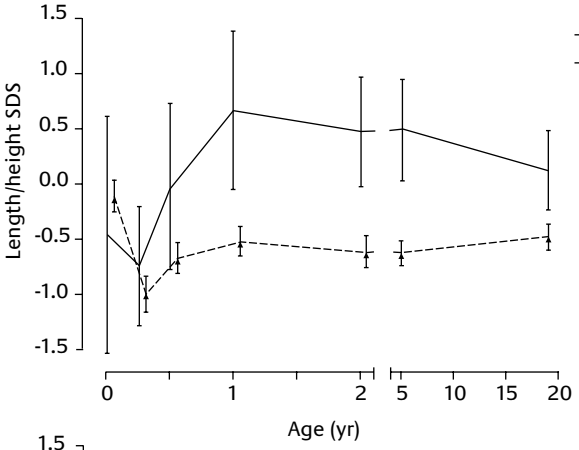

Figure B. Men

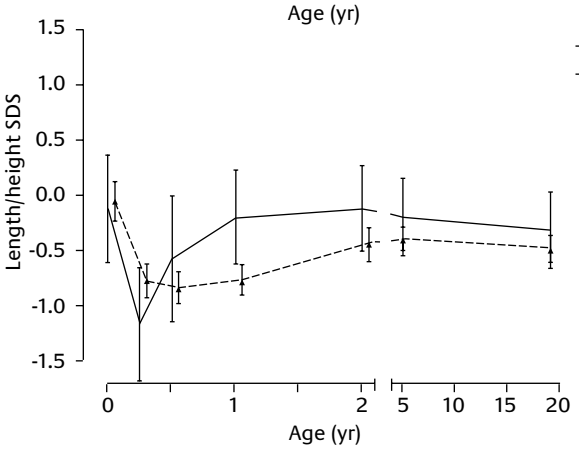

Figure C. Women

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10 Could cortisol explain the association between birth weight and cardiovascular disease in later life?:

a meta-analysis

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Abstract

Objectives:

Studies about the association between birth weight and circulating cortisol level have been published from 1998 onwards. However, their findings were inconsistent. To quantitatively assess the overall association between birth weight and circulating cortisol level, we aimed to perform a meta-analysis of the published literature.

Methods:

A literature search was conducted in PubMed, and selected papers were systematically reviewed. A pooled regression coefficient was calculated for the entire group as well as for males and females separately.

Results:

Data from 11 study populations were pooled (N=2,301). These populations differed with respect to geographical area, age, sex distribution, inclusion criteria, and gestational age. We found a statistically significant inverse association between birth weight and circulating cortisol level: a 1 kg lower birth weight was associated with a 25.3 (95% CI: 5.9 to 44.8) nmol/l higher cortisol level. Separate results were reported for males and females in 6 study populations. The association in males was 20.6 (95% CI: 4.2 to 37.0) nmol/l per kg and in females it was 30.9 (95% CI: 7.4 to 54.4) nmol/l per kg.

Conclusions:

Differences between study populations hampered the comparability of the included studies. Although the majority of studies were underpowered, by using a meta-analytic approach we found an inverse association between birth weight and circulating cortisol level. Thus, our findings suggest that there is some evidence for a possible role of the hypothalamus-pituitary-adrenal axis in the epidemiological association between birth weight and cardiovascular disease. However, the strength of the overall association between birth weight and circulating cortisol level was weak.

Introduction

There is evidence from epidemiological studies that cardiovascular disease – including its risk factors, such as hypertension and type 2 diabetes – is associated with low birth weight (1-3). The link between cardiovascular disease and low birth weight might be explained by a phenomenon called perinatal programming, i.e. persistent structural, hormonal, and/or metabolic adaptations of an individual in response to specific insults acting at critical periods in development. Alternatively, it might be explained by genes which predispose to intrauterine growth retardation as well as to cardiovascular disease and type 2 diabetes (4).

Pathologically increased activity of the hypothalamus-pituitary-adrenal (HPA) axis – as in Cushing's syndrome – is associated with cardiovascular disease, raised blood pressure, and impaired glucose tolerance. More subtle activation of the HPA axis is associated with a similar but milder phenotype (5-8). Through its effects on several cardiovascular, hormonal, and metabolic targets, and its possible susceptibility to the effects of perinatal programming (9), in the early 1990s the idea was launched that the HPA axis may explain part of the epidemiologic association between birth weight and later cardiovascular disease (10). Evidence for possible programming of the HPA axis in humans was first suggested in 1996 by Clark et al, who found a U-shaped relation between birth weight and glucocorticoid metabolite excretion in 24-h urine samples (11).

In 1998, Phillips et al were the first to report an inverse association between birth weight and circulating cortisol level in a population of elderly men (12). Thereafter, a number of other studies on this topic were published. As these had different study populations, methods, and results, we systematically reviewed the available literature. We conducted a meta-analysis in order to investigate whether there is really an inverse association between birth weight and circulating cortisol level.

Methods

A literature search was conducted for papers published between January 1995 and June 2004 in PubMed. Papers about cortisol in blood in relation to birth weight were searched using combinations of the text words "birth weight", "birthweight", "cortisol", and "hydrocortisone" in title or abstract. We restricted the search to studies in humans and written in English. Papers were identified by title and selected by abstract reviewing. Papers were selected if the abstract indicated that basal cortisol in blood (plasma or serum) had been measured in relation to birth weight in persons aged >1 years. Reference lists of selected papers were searched for further relevant studies. For completeness, a literature search in EMBASE was also performed.

The first 2 authors independently reviewed the selected papers. Of the included papers, the following characteristics were recorded: year of publication and sample size, and sex distribution, age, gestational age, birth weight, and cortisol level of the participants, and the type of cortisol assay.

Table 1. Characteristics of included studies by year of publication.

Reference number	First author	Publication year of study	Geographical area	Birth weight groups	Sex	N/males	Age (yrs)	Gestational age (weeks)	Birth weight (kg)	Cortisol (nmol/l)
12	Phillips	1998	Hertfordshire, UK	-	M	370/370	59 to 70	NR	NR	344.0±112.0
21	Dahlgren	1998	Göteborg, Sweden	SGA[a]	M/F	53/41	2 to 14	38.9±2.0	NR*	444.0±138
				AGA[a]	M/F	131/93	3 to 15	39.5±1.9	NR*	481.6±NR‡
23	Houang	1999	Paris, France	IUGR[b]	M/F	40/20	1.1 to 13.5	38.5±1.8	2.48±0.51	316.3±147.0
				Normal[b]	M/F	26/13	0.9 to 13.6	39.5±1.0	3.20±0.18	262.6±150.0
17	Phillips	2000	Hertfordshire, UK	-	F	306/0	60 to 71	NR	3.40±0.52	350.0±127.6
17	Phillips	2000	Preston, UK	-	M/F	199/92	45 to 54	NR	3.20±0.71	412.5±182.0
17	Phillips	2000	Adelaide, Australia	-	M/F	165/87	20	NR	3.48±0.64	383.5±192.7
16	Levitt	2000	Cape Town,	UFA[c]	M/F	36/20	20	39.3±0.8†	2.35±0.22†	484.9±166.3
			South Africa	AFA[c]	M/F	32/15	20	39.3±0.9†	3.05±0.21†	418.6±160.6
18	Szathmári	2001	Budapest, Hungary	LBW[d]	M/F	70/37	20	33.1	1.81±0.38	260.6±65.0
				Normal[d]	M/F	30/16	20	39.5	3.28±0.37	210.7±65.0
15	Kajantie	2002	Helsinki, Finland	-	M/F	421/157	65.1 to 75.8	39.9±1.3	3.41±0.42	384.2±137.0
19	Tenhola	2002	Kuopio, Finland	SGA[e]	M/F	55/20	12	39.0±1.4	2.45±0.32	292.5±217.4¶
				AGA[e]	M/F	55/20	12	39.7±1.5	3.46±0.48	272.1±217.4¶
20	Walker	2002	Edinburgh, UK	AGA[f]	M/F	19/9	22 to 25	32.0±0.8	1.67±0.22	210±165.6§
				IUGR[f]	M/F	15/4	22 to 25	35.2±1.3	1.70±0.21	158±121.2§
				Normal[f]	M/F	27/11	22 to 25	40.1±1.7	3.13±0.44	191±227.6§
22	Herrick	2003	Lanarkshire, UK	-	M/F	251/119	28 to 32	38.9±1.3	3.05±0.40	390.0±145.9

Gestational age, birth weight, and cortisol are shown as mean±SD.

NR: not reported.

* Difference in g between groups estimated from difference in SDS.

† Cortisol data came from a subset of 68 subjects of a larger study population (N=137), whereas data on birth weight and gestational age came from the entire population.

‡ SD could not be calculated because of an impossible value in the paper. We made the assumption that the SD of the AGA group was similar to the SD of the SGA group, which was calculated from the interquartile range.

¶ SD estimated from P value.

§ Value estimated from graph.

Definitions of birth weight groups as reported in the papers:

[a] SGA: short or light for gestational age; <-2 SD in height at 2 years of age.

AGA: appropriate-for-gestational-age; short healthy children (N=75) and healthy children with heights within the normal range (±2 SD) (N=56).

[b] IUGR: intrauterine growth retardation; birth length for gestational age ≥2 SD below the population mean.

Normal: without intrauterine growth retardation.

[c] UFA: underweight for gestational age; birth weight ≤10th percentile.

AFA: appropriate weight for gestational age; birth weight between 25th and 75th percentiles.

[d] LBW: low birth weight; gestational age ≤36 weeks and birth weight <2,500 g.

Normal: gestational age ≥38 weeks and birth weight ≥2,500 g.

[e] SGA: small-for-gestational-age; birth weight and/or length and/or ponderal index for gestational age >2 SDS below the population mean.

AGA: appropriate-for-gestational-age; birth weight and/or length and/or ponderal index for gestational age ≥-2 and ≤2 SDS of the population mean.

[f] AGA: appropriate-for-gestational-age; birth weight <2,000 g and >10th percentile.

IUGR: intrauterine growth retardation; birth weight <2,000 g and <10th percentile.

Normal: birth weight >2,000 g.

Statistical analysis

If possible, regression coefficients and their standard errors were directly extracted from the papers. In several papers only the mean circulating cortisol level with standard error or SD was displayed for subgroups of birth weight. In this situation, the regression coefficient was estimated by:

$$b = \frac{\sum n_i (X_i - X)(Y^i - Y)}{\sum n_i (X_i - X)^2},$$

with $\overline{Y}_i$ the mean circulating cortisol level in category i, $\overline{X}_i$ the mean birth weight, and n_i the number of subjects in category i, and with $\overline{X} = \sum n_i \overline{X}_i / \sum n_i$ the estimated overall mean birth weight and $\overline{Y}_i = \sum n_i \overline{Y}_i / \sum n_i$ the estimated overall mean circulating cortisol level.
The standard error of b was then estimated by:

$$se(b) = \frac{\sqrt{\sum n_i^2 (\overline{X}_i - \overline{X})^2 se_i^2}}{\sum n_i (\overline{X}_i - \overline{X})^2},$$

with se_i the standard error of Y in category i.
Regression coefficients of individual studies were pooled using techniques for meta-analysis (13). To take account of possible heterogeneity between studies, a meta-analysis with random study effect was performed.

Results

Description of the included studies

The primary PubMed search yielded 183 papers. The restriction to studies in humans and written in English limited the search result to 144 titles. Of these, 24 were selected from the abstract. Nine of these were included after having read the full content (12;14-21). One study was conducted in 3 populations of different ages in cohorts from Hertfordshire (UK), Preston (UK), and Adelaide (Austrialia) (17); one of these populations (the Hertfordshire cohort) was the females of a cohort of which the males had been analyzed earlier with respect to circulating cortisol level (12). One study (14) had included the men and women from the same cohort as had been previously studied by Phillips et al (12;17), and was therefore excluded. In addition, another 2 papers (22;23) were included after having examined the reference lists of the already included papers. The search in EMBASE did not identify any additional relevant papers. Thus, our analysis was based upon 10 papers with the data of 2,301 subjects from 11 study populations.

Table 1 shows the characteristics of the included studies. Sample sizes of the individual study populations ranged from 61 to 421. The majority of studies were performed in Europe.

Although all study populations were mixed, separate results for males and females were reported for 6 study populations (12;15;17;18;20). Inclusion criteria differed substantially between studies. Two studies had included individuals born prematurely (18;20). Studies used different definitions for low birth weight. Mean gestational age ranged from 32.0 to 40.1 weeks, and mean birth weight from 1.67 to 3.48 kg. Mean circulating cortisol level ranged from 158.0 to 481.6 nmol/l. In most studies, cortisol was analyzed in a single venous blood sample drawn between 7:30 and 10:00 h after an overnight fast. In 1 study, an alternative procedure was performed: children were kept in hospital for at least a 24-h period during which they received a normal diet (21). Within the 24 hours, 8 samples were drawn. The mean circulating cortisol level of the samples drawn at 6.00 and 10.00 h was used in the meta-analysis. Cortisol was analyzed by radioimmunoassay in 7 studies (12;17;18;20-23), other immunoassays in 2 studies (15; 19), and by an "ACS auto analyzer" in 1 study (16).

In 5 study populations, a statistically significant inverse association between birth weight and circulating cortisol level was found in males and/or females, or in the population as a whole (12;16-18). To express the relation between birth weight and circulating cortisol level, either linear regression analysis (12;17) or comparison of mean circulating cortisol level between subgroups of birth weight were used by the studies (15;16;18-23). To estimate the regression coefficient from the paper by Tenhola et al (19), data on the mean birth weight of the small-for-gestational-age (SGA) and appropriate-for-gestational-age (AGA) groups were extracted from a previous study by the same research group (24). The standard error of the cortisol values was estimated from the P value of the t test for the difference between the SGA and AGA group. In the paper by Dahlgren et al (21), birth weight was displayed as SD score (SDS) only. To estimate the difference between the SGA and AGA groups in gram, the intrauterine growth curve of the Swedish reference population was used (25). Reported (12;17) and estimated (15;16;18-23) regression coefficients with 95% and 99% CIs of the individual study populations are summarized in Table 2.

Meta-analysis

A 1 kg lower birth weight was associated with a 25.3 (95% CI: 5.9 to 44.8) nmol/l higher circulating cortisol level (Figure 1). In comparison, the association was 27.9 (95% CI: 17.0 to 38.6) nmol/l per 1 kg in a fixed effects model. In one of the papers by Phillips et al (17), only regression coefficients adjusted for age and BMI were displayed. Therefore, an analysis was also performed after exclusion of the 3 study populations in their paper. This strongly reduced the strength of the association to 18.5 (95% CI: -12.7 to 49.7) nmol/l per kg. Furthermore, an analysis was performed after exclusion of individuals born prematurely (18;20). The strength of the association between birth weight and circulating cortisol level hardly changed: 24.2 (95% CI: -0.6 to 48.9) nmol/l per kg.

To test whether the association between birth weight and circulating cortisol level was different between genders, we also performed an analysis on the data of the 5 papers (6 study

populations) that displayed data for males and females separately (12;15;17;18;20). A 1 kg lower birth weight was associated with a 20.6 (95% CI: 4.2 to 37.0) nmol/l higher circulating cortisol level in males (Figure 2A), and a 30.9 (95% CI: 7.4 to 54.4) nmol/l higher cortisol level in females (Figure 2B).

We also studied the relation between the sample size of each study population, and the strength of the association between birth weight and circulating cortisol level. The strength of the association within study populations was irrespective of the sample size (Figure 3).

Discussion

We performed a systematic review of the available literature about the association between birth weight and circulating cortisol level at later age. Although the majority of studies included in our review did not find an effect of birth weight on circulating cortisol level, we found a statistically significant inverse association in a pooled data analysis.

It should be remarked that differences between study populations hampered the comparability of the included studies. First, the included study populations differed in geographical area. It has been demonstrated that there is a small difference in circulating cortisol level between white and black persons (26), but the majority of studies included in our review were performed in Europe. Only 1 study, which was conducted in South Africa, had included the children of "primigravid women of mixed ancestry" (16). Second, there were large differences in age between the included study populations. As has been demonstrated by others, physiological ageing is associated with a reduced amplitude of circadian cortisol fluctuations and altered negative feedback control, but morning circulating cortisol level does not seem to

Table 2. Regression coefficients with 95% and 99% confidence intervals of included study populations.

First author of study	ß	95% CI		99% CI	
		lower limit	upper limit	lower limit	upper limit
Phillips, Hertfordshire	21.9	5.5	38.2	0.3	43.4
Dahlgren	-44.8	-94.3	4.8	-109.9	20.4
Houang	32.7	-68.5	133.8	-100.4	165.8
Phillips, Preston	41.3	4.3	78.3	-7.5	90.0
Phillips, Adelaide	36.3	4.0	68.6	-6.3	78.8
Levitt	76.3	25.3	127.3	9.1	143.5
Szathmári	32.8	14.1	51.5	8.2	57.4
Kajantie	-10.7	-50.1	28.7	-65.5	41.2
Tenhola	20.2	-60.3	100.7	-85.7	126.1
Walker	-1.9	-50.0	46.2	-65.2	61.4
Herrick	1.2	-49.7	52.2	-65.8	68.3

A positive regression coefficient indicates an inverse relation between birth weight and circulating cortisol level.

change much with age (27). Third, there were differences in the sex distribution between the included study populations. As differences in A-ring reduction between genders have been reported (28), data were also analyzed separately for males and females. However, we did not find evidence that the association between birth weight and circulating cortisol level was substantially different between genders. This is in line with the findings from a recent study performing dynamic HPA axis function testing, which found that the strength of the relation between birth weight and plasma cortisol rise after adrenocorticotrophin (ACTH) 1-24 was similar for men and women (29). Fourth, studies used different definitions for low birth weight. Finally, 2 studies had included individuals born prematurely. As there is evidence that circulating cortisol level as well as cortisol production are dependent on gestational age (15;20), we also performed an analysis restricted to individuals born after term gestation. However, exclusion of (only a limited number of) individuals born prematurely hardly changed the association between birth weight and circulating cortisol level. As we believe that the 5 aforementioned differences between study populations could not be ignored, a meta-analysis with random study effect was performed, though the strength of the overall association was nearly the same in a fixed effects model.

Studies also differed in their analytical methods: either linear regression analysis or comparison of mean circulating cortisol level between subgroups of birth weight. If the latter method was used – which was the case for most studies – it was always necessary to estimate the regression coefficient and the standard error. In one of the studies by Phillips et al (17), only

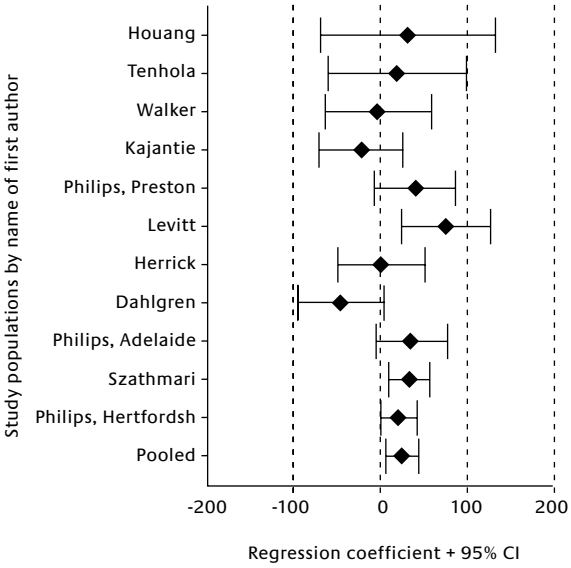

Figure 1. Individual and pooled regression coefficients with 95% confidence intervals.

Increase in circulating cortisol level (nmol/l) for each 1 kg lower birth weight with 95% CI (X axis), displayed for each study population (Y axis, by name of first author, ordered by CI width), and for the pooled data.

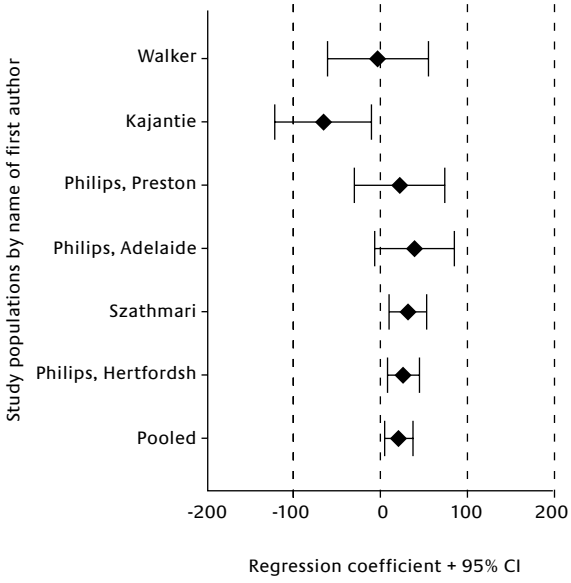

Figure 2. Individual and pooled regression coefficients with 95% confidence intervals – analyses by gender.

Increase in circulating cortisol level (nmol/l) for each 1 kg lower birth weight with 95% CI (X axis), displayed for each study population where data were provided by gender (Y axis, by name of first author), and for the pooled data.

Figure 2A. Males

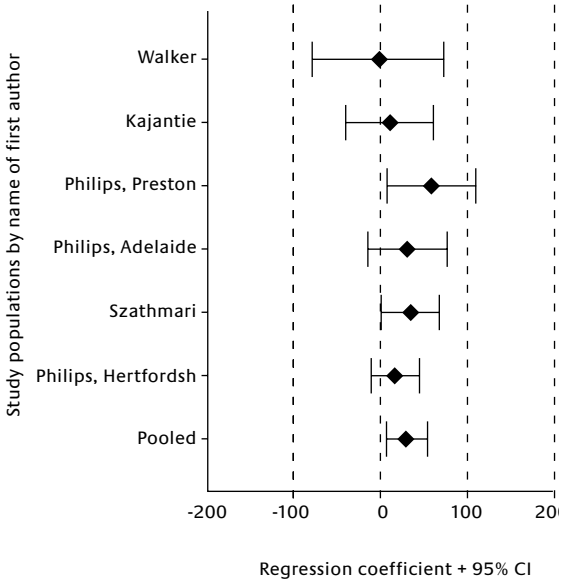

Figure 2B. Females

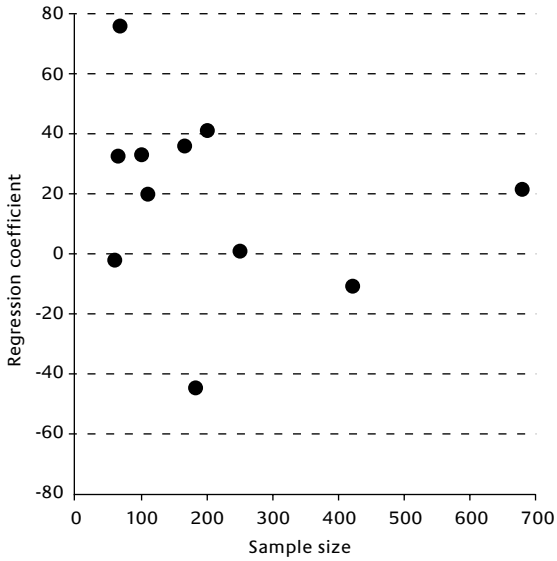

Figure 3. Funnel plot: regression coefficient versus sample size of the 11 included study populations.

The number of individuals within each study population (X axis) is plotted against its regression coefficient (Y axis).

adjusted data were reported (adjusted for age and BMI). As there are constraints in adjusting for current size in "fetal origins" studies (30), we also performed an analysis after exclusion of their data. This strongly reduced the magnitude of the pooled regression coefficient. However, earlier studies in the same subjects from Hertfordshire showed a positive relation between birth weight and current BMI in the males (31), and no relation at all in the females (32). Furthermore, current BMI was inversely associated with circulating cortisol level. Thus, adjustment for current BMI would rather decrease the relation between birth weight and circulating cortisol level than enhance it, implying that we only underestimated the true effect of birth weight on cortisol. Therefore, we did not exclude their data from our meta-analysis.

Our meta-analysis was based upon published data only. There may be a tendency to selectively publish results from large studies that are statistically significant. However, most studies included in our analysis were negative, i.e. they showed no statistically significant relation between birth weight and circulating cortisol level. Moreover, we did not find an association between the sample size of each study population and the strength of the association between birth weight and circulating cortisol level. We therefore believe that there is only minimal influence of possible publication bias on the outcomes of our meta-analysis.

How could the inverse association between birth weight and circulating cortisol level be explained? Circulating cortisol reflects the balance between cortisol production, and reversible interconversion to cortisone by 11ß-hydroxysteroid dehydrogenases (11ß-HSDs) and irreversible breakdown by A-ring reductases. Elevated circulating cortisol level may therefore result from increased cortisol production as well as decreased inactivation. The elderly males from

Hertfordshire with low birth weight had elevated 24-h urinary excretion of cortisol metabolites and enhanced responses of plasma cortisol to ACTH 1-24 (33). Their cortisol and ACTH levels after overnight low-dose dexamethasone suppression did not differ from the other men (33;34). Unexpectedly, their ACTH and cortisol responses to corticotrophin releasing hormone after dexamethasone were blunted rather than enhanced (34). Similar to the men, the females from Hertfordshire with low birth weight had enhanced cortisol responsiveness to synthetic ACTH (29). There is no evidence in humans that low birth weight is associated with alterations in activities of 11ß-HSDs (11). Interestingly, however, in line with earlier findings in rats (35), a recent study in small preterm infants showed that reduced placental 11ß-HSD type 2 activity was associated with less cortisone relative to cortisol in cord blood and lower birth weight (36).

Conclusions

Papers about the association between birth weight and basal cortisol level have been published from 1998 onwards. Sources of heterogeneity hampered the comparability of these studies. Although the majority of studies were underpowered, by using a meta-analytic approach we found an inverse association between birth weight and circulating cortisol level. Thus, our findings suggest that there is some evidence for a possible role of the HPA axis in the epidemiological association between birth weight and cardiovascular disease, at least in persons born after term gestation, but it is emphasized that the strength of the overall association between birth weight and basal cortisol level is weak.

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11 General discussion

Summary of the main findings

Effect of perinatal growth retardation on long-term height gain

According to current legislation, children born small-for-gestational-age (SGA) who fail to show postnatal catch-up growth are candidates for growth hormone (GH) therapy (1;2). At present, very preterm infants born appropriate-for-gestational-age (AGA) who grow poorly as neonates, resulting in an "SGA condition at term", are excluded from GH therapy if their small size evolves toward a short stature in childhood. Chapter 3 shows that the growth patterns of very preterm infants born SGA and those born AGA with a small size near term are virtually indistinguishable from the age of 3 months post-term onwards and, thus, extension of the SGA indication for GH therapy with short children with a history of neonatal growth retardation after preterm birth is recommended, provided the results are monitored until such extension is conclusively validated.

Effects of prematurity per se on the adult metabolic profile

Recently, it was found that 7-year-olds born prematurely were more insulin-resistant, assessed with an intravenous glucose tolerance test, than age-matched normal controls (3). In line with these observations, chapter 6 shows that the survivors of very preterm birth were relatively insulin-resistant at age 19 years. Furthermore, chapters 4 and 8 show that these subjects had a more central pattern of fat distribution and higher blood pressure, respectively. The clustering of cardiovascular risk factors in survivors of very preterm birth resembles the effects of permanent activation of the hypothalamus-pituitary-adrenal (HPA) axis, analogous to persons born with a lower birth weight (chapter 10). In prematurely born subjects, this may have contributed to improved neonatal survival, or, alternatively, it may be a consequence of the very preterm birth. Chapter 8 suggests that this phenotype may, in part, result from selective survival, because insulin resistance and adequate blood pressure regulation could optimize the fitness of the very preterm newborn. Insulin resistance may offer protection against neuroglycopenia, and raised blood pressure may, in the absence of an adequare cerebral autoregulation, prevent cerebral hypoperfusion, potentially life-threatening conditions which are commonly observed in the early neonatal course of very preterm infants. Chapter 9 shows that carriers of the 23K variant in the glucocorticoid receptor gene, associated with a mild glucocorticoid resistance (4;5), had a normal stature during childhood and in adulthood, and were less insulin-resistant than non-carriers. This suggests that the 23K variant protects against postnatal growth failure and insulin resistance after very preterm birth. Whatever the explanation, selective survival or causation, the price of neonatal survival after very preterm birth is to be paid in later life.

Effects of early growth on the adult metabolic profile

Effects of early growth on the risk, in the general population, of developing cardiovascular disease and type 2 diabetes are well described. However, at the start of the studies presented

in this thesis, little was known about the effects of early growth on the propensity to these diseases after preterm birth. Analogous to persons born after a term pregnancy, the path of early growth contributes to the onset of metabolic disease in very preterm subjects. Chapter 6 shows that overweight subjects after a lower birth weight for gestational age were more insulin-resistant at age 19 years than subjects with a similar fat mass after a higher birth weight. It has been argued for term individuals that the metabolic changes associated with low birth weight are usually insufficient to produce an increased risk of disease. Rather, a second hit, such as greater fat mass, is required for developing insulin resistance. According to Reaven et al, insulin resistance (hyperinsulinaemia) is fundamental in the development of many of the features of the metabolic syndrome (6). In our studies, early growth was unrelated to blood pressure, the serum lipid profile, or carotid intima-media thickness at age 19 years (chapters 5 and 7), probably because it takes many years more for these sequelae to develop after a prolonged period of hyperinsulinaemia. Therefore, continuing follow-up of the Project On Preterm and Small-for-gestational-age infants (POPS) cohort, as well as other preterm populations, is warranted.

Methodological considerations

Selection bias

In the POPS-19 study, selection bias could have been introduced at different levels. First, in 1983, there may have been a difference between participating and non-participating hospitals in the quality of the neonatal care provided. However, the far majority of Dutch hospitals were involved in the cohort retrieval in 1983, leading to the inclusion of 94% of all very preterm and/or very-low-birth-weight infants born in that year. Second, 28% of children were deceased before the age of 19 years (7), of whom were the majority (27%) in the first year (8). In the original cohort, in-hospital mortality was strongly related to gestational age and, accordingly, to the incidence and severity of the respiratory distress syndrome (8). As suggested by chapter 8, it seems plausible that there is a possible link between factors associated with neonatal mortality and some outcomes at age 19 years, such as blood pressure and parameters of insulin resistance. Third, the response at 19 years was 62%. Non-response was associated with male gender, non-white ethnicity, and lower socio-economic status but it was unrelated to gestational age or birth weight (9). A possible relationship between birth weight and the adult outcomes would be concealed if the lower birth weight subjects with a high risk of disease selectively declined to participate. This seems not very likely.

Confounding

It has been argued that the relation between lower birth weight and the adult metabolic profile is due to confounding by socio-economic status (10;11), since maternal smoking is associated

with lower birth weight of her offspring, and, on the other hand, smoking, drinking and eating habits, and physical exercise influence the propensity to cardiovascular disease and type 2 diabetes. A sedentary life style is more common among persons with a lower socio-economic status. However, in the studies described in this thesis adjustment for parental socio-economic status did not alter the strength of the associations between early growth and the adult outcomes, implying that confounding by socio-economic status is not likely to explain the observations.

Inappropriate statistical adjustment for current size

Early size predicts later size. In our population, it was found that birth weight SDS was positively associated with BMI and fat-free mass at age 19 years (chapter 4). Also, weight gain in infancy was positively associated with BMI, and absolute and relative fat mass, and abdominal fat distribution. Adult fatness, in turn, is always positively associated with insulin resistance and blood pressure. In the past, adjusting for current size has been justified on the grounds that birth weight is positively related to later size, and later fatness to the outcome of interest, and if not adjusted for could obscure a negative relation between birth weight and the outcome variable (12). Tu et al examined with computer simulations the impact of adjusting for different correlations between current weight and birth weight and between current weight and blood pressure to assess their impact on associations between birth weight and blood pressure (13). Regardless of the direction of association between birth weight and blood pressure (positive, inverse or absent), adjustment for current weight created or exaggerated inverse associations between birth weight and blood pressure ("reversal paradox"). Therefore, the analyses presented in this thesis were not corrected for current size.

External validity

Due to improved perinatal care, especially the widespread application of maternal glucocorticoid treatment for impending preterm delivery and the introduction of synthetic surfactant, neonatal mortality of very preterm infants has improved dramatically between 1983 and 1996-1997: from 30 to 11% (14). The sicker individuals survive nowadays. This increasing survival rate has resulted in greater numbers with respiratory failure necessitating mechanical ventilation (14). Ventilated infants occasionally need dexamethasone treatment to wean them off the ventilator. Short- and possibly also long-term height gain is impaired in dexamethasone-treated infants (15-17). Furthermore, it can be inferred from animal experiments that high-dosed dexamethasone has the potential to produce life-long deleterious effects on HPA axis activity and cardiac function (18;19). Improved survival has also led to a rising incidence of bronchopulmonary dysplasia (14). Obviously, for those reasons, the results in this thesis may not be fully representative for the current generation of prematurely born children.

Relevance and implications

From a public health perspective, our primary attention should focus on reducing the number of preterm births. Factors amenable to intervention are older maternal age at first birth, now on average 29.4 years in The Netherlands (20), and maternal smoking.

In situations where preterm delivery cannot be avoided, a single course of maternal betamethasone treatment is effective in reducing neonatal mortality and the incidence of the respiratory distress syndrome and related conditions (21). Fear for possible long-term side effects of this therapy is not necessary. Previous studies have demonstrated that there were no differences between betamethasone exposed and unexposed subjects in later lung function, cognitive performance, psychiatric morbidity, and psychosexual development (22;23). We have added that maternal betamethasone 24 mg does not adversely influence the adult metabolic health in her offspring. Thus, obstetricians should continue to use a single course of betamethasone for the prevention of the neonatal respiratory distress syndrome.

Very preterm infants with neonatal growth retardation grow in a way that has previously been described for children born SGA (24;25). These long-term findings strengthen the plausibility of extending the SGA indication for GH therapy in such a way that this group of preterm infants are no longer excluded if they have a persistent short stature. However, the effectiveness of GH therapy remains to be explored in such children.

Like full-term children born SGA, very preterm infants are at risk for developing (abdominal) obesity, type 2 diabetes, and hypertension. Very preterm infants born SGA who become overweight as young adults have the greatest risk for developing type 2 diabetes. As obesity tracks from childhood to young adulthood (26), paediatricians and primary health care workers should be aware of fat accumulation in the follow-up of very preterm infants, especially of those born SGA. Weight reduction should be advised, though it is unclear whether this can reverse the metabolic alterations associated with very preterm birth. As the expression of type 2 diabetes and hypertension is strongly dependent on life style factors, physical exercise should be promoted and smoking be discouraged.

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12

Summary

Children born small-for-gestational-age (SGA) are at risk for short stature, and cardiovascular disease and type 2 diabetes mellitus in later life. There is some preliminary evidence for a similar phenotype in survivors of preterm birth, but due to lack of long-term follow-up of preterm populations it is unknown whether the observed associations in childhood persist into adult life. We tested in 19-year-olds born before 32 gestational weeks from the Project On Preterm and Small-for-gestational-age infants (POPS) cohort the effect of early growth on subsequent height development and the adult metabolic profile.

The POPS cohort started in 1983 and comprised of 94% of the very preterm (<32 weeks' gestation) and/or very-low-birth-weight (<1,500 g) infants born in The Netherlands in that year. The study's main objective was to study general and disease-specific mortality of such infants. From birth onwards, follow-up was continued which enabled to study handicaps, cognitive performance, linear growth, and various other characteristics. In 1999, an initiative was launched to study the POPS cohort at young adult age. Assessments took place between April 2002 and May 2003, at 19 years of age. The response rate was 62%.

Height, weight, and abdominal and hip circumferences were measured according to standard procedures. Values were transformed to SD score (SDS) in order to allow comparison with population references (Fourth Dutch Growth Study). Skinfold thicknesses were measured at 4 locations, which enabled us to calculate fat mass and fat-free mass. Furthermore, venous blood was drawn fasted for determination of glucose, insulin, C-peptide, and cholesterol concentrations, and blood pressure and carotid intima-media thickness (CIMT) were measured.

In chapter 2, we discussed the current SGA indication for growth hormone (GH) therapy. Preterm infants with a stormy postnatal course grow poorly as neonates. This is attributed to a combination of factors, including respiratory distress syndrome necessitating assisted ventilation, low energy intake, infections, and postnatal glucocorticoid treatment. This may result in an "SGA condition at term age". According to current legislation, these children are excluded from GH therapy if their small size at term evolves toward a short stature in childhood. The only change that we propose is that term is taken as the measuring stick for assessing whether a short child is eligible for GH therapy.

The growth pattern up till adulthood of very preterm infants is presented in chapter 3. Three groups were differentiated: SGA children, appropriate-for-gestational-age (AGA) children who were small at 3 months post-term (labelled preterm-growth-restraint, PGR), and AGA children who remained appropriate-sized (non-PGR). We found that childhood growth and final height were similar in SGA and AGA PGR children. Adult stature in these groups was -1.1 to -1.2 SDS, whereas in AGA non-PGR children it was -0.4 SDS. Groups did not differ in target height SDS. AGA PGR children who were still short at 5 years of age had a similar risk, approximately 90%, as SGA children to become short as adults.

Chapter 4 shows that prematurely born adults had an abdominal circumference and a waist-to-hip ratio much greater than the population reference mean, especially women. Birth weight was positively associated with adult BMI SDS and fat-free mass. Both weight gain between birth and the age of 3 months post-term, and between 3 months and 1 year post-term were positively related to BMI SDS, and absolute and relative fat mass, and abdominal circumference SDS.

In chapter 5, we addressed the effects of birth weight and postnatal weight gain on the serum lipid profile and CIMT. Birth weight and postnatal weight gain were unrelated to these outcomes. Rather, strong associations between indices of current body composition and the serum lipid profile were found, as well as between lipid profile and CIMT.

The effects of birth weight and postnatal weight gain on parameters of insulin resistance are described in chapter 6. Prematurely born adults were found to be relatively insulin-resistant. Insulin resistance was measured with fasting levels of insulin and C-peptide as well as with the homeostatic model assessment for insulin resistance index (HOMA-IR). HOMA-IR is calculated as fasting glucose x insulin / 22.5 and approximates to 1 in young non-obese persons if glucose is measured in mmol/l and insulin in mU/l. Weight gain between birth and the age of 3 months post-term was weakly associated with higher levels of fasting insulin, but it was not associated with the other parameters of insulin resistance. Adult fatness was positively associated with all parameters of insulin resistance. The effect of absolute and relative fat mass on these parameters was dependent on its interaction with birth weight SDS.

A high prevalence of hypertension, i.e. a blood pressure >140/90 mmHg according to the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure VII criteria, was found among prematurely born adults and is presented in chapter 7. Birth size and postnatal growth were unrelated to blood pressure or hypertension.

A single course of maternal glucocorticoid treatment is effective in reducing neonatal mortality after preterm birth. However, in animals, maternal glucocorticoid treatment is associated with lifelong hyperglycaemia and hypertension, and impaired nephrogenesis in offspring. Findings from studies in humans on this topic are highly contradictory due to a number of methodologic flaws and renal function after glucocorticoid exposure has never been assessed. In chapter 8, we assessed the effect of maternal betamethasone 24 mg on the adult metabolic profile, including renal function, of her offspring. We did not find any long-term adverse effects of antenatal betamethasone, with the exception of an effect on glomerular filtration rate (GFR) in women. In women, GFR was lower after betamethasone: -7.4 (95% CI: -13.3 to -1.5) ml/min per 1.73 m^2. Although the reduction in GFR was not clinically relevant at 19 years, it might predict an increased risk of chronic renal failure in prematurely born women who were exposed antenatally to betamethasone.

A previous study in a small population of young adults has suggested that basal cortisol levels are elevated after preterm birth. Chapter 9 investigated the effect of the R23K and N363S polymorphisms in the glucocorticoid receptor gene, associated with sensitivity to cortisol, on

linear growth and the adult metabolic profile. 23K carriers had lower fasting insulin levels and HOMA-IR than non-carriers. Furthermore, in contrast to non-carriers, 23K carriers showed complete catch-up growth between ages 3 months and 1 year post-term and attained height was similar to the population reference mean. The N363S polymorphism was not associated with any of the outcomes. Thus, carriers of the 23K variant are, at least in part, protected against postnatal growth failure and insulin resistance after preterm birth.

Chapter 10 is a meta-analysis of published reports on the association between birth weight and basal cortisol level. A literature search yielded 10 reports (11 study populations) with the data of 2,301 subjects. Differences between study populations and analytical methods hampered the comparability of studies. After pooling of the data, we found a statistically significant inverse association between birth weight and circulating cortisol level: a 1 kg lower birth weight was associated with a 25.3 (95% CI: 5.9 to 44.8) nmol/l higher cortisol level. These findings suggest that there is some evidence for a possible role of the hypothalamus-pituitary-adrenal axis in the epidemiologic association between birth weight and cardiovascular disease.

Samenvatting

Kinderen die small-for-gestational-age (SGA) geboren zijn, hebben een verhoogd risico op kleine lichaamslengte, cardiovasculair lijden en diabetes mellitus type 2. Er is enig bewijs voor een soortgelijk fenotype in overlevers van prematuriteit, maar door gebrek aan lange-termijn follow-up is het onbekend of de tot dusver gevonden associaties op de kinderleeftijd in het volwassen leven persisteren. Wij testten in 19-jarigen die voor 32 zwangerschapsweken geboren waren uit het Project On Preterm and Small-for-gestational-age infants (POPS) -cohort het effect van vroege groei op de lengtegroei en het metabole profiel.

Het POPS-cohort werd geformeerd in 1983 en omvatte 94% van alle kinderen in Nederland uit dat jaar die zeer prematuur (<32 zwangerschapsweken) en/of met een zeer laag geboortegewicht (<1.500 g) geboren waren. Het hoofddoel van de studie was het bestuderen van de algemene en ziekte-specifieke mortaliteit van dergelijke kinderen. Na de geboorte werd de follow-up gecontinueerd, waardoor latere handicaps, cognitief functioneren, groei en vele andere eigenschappen bestudeerd konden worden. In 1999 werd een initiatief ondernomen om het POPS-cohort op jongvolwassen leeftijd te bestuderen. De metingen vonden plaats tussen april 2002 en mei 2003, op de leeftijd van 19 jaar. De respons was 62%.

Lengte, gewicht, en taille- en heupomtrek werden gemeten volgens standaard procedures. Waarden werden getransformeerd naar SD score (SDS) teneinde te kunnen vergelijken met Nederlandse normgegevens (Vierde Landelijke Groeistudie). Huidplooidikten werden op 4 locaties gemeten, wat ons in staat stelde om de vetmassa en de vetvrije massa te berekenen. Verder werd veneus bloed afgenomen in nuchtere toestand ter analyse van glucose, insuline, C-peptide en cholesterol, en werden de bloeddruk en intima-media dikte van de carotiden (CIMT) gemeten.

In hoofdstuk 2 bediscussieerden wij de huidige SGA-indicatie voor groeihormoon- (GH-) behandeling. Prematuren met een stormachtig postnataal beloop groeien matig als neonaten. Dit is te toe te schrijven aan een combinatie van factoren, waaronder respiratoir falen, lage calorische inname, infecties en postnatale glucocorticoïdtherapie. Dit kan resulteren in een "SGA-status op a terme datum". Volgens de huidige richtlijnen zijn deze kinderen uitgesloten van GH-behandeling als hun kleine gestalte op de uitgerekende datum leidt tot een kleine gestalte op de kinderleeftijd. De enige verandering die wij voorstellen is dat de a terme datum als meetmoment wordt genomen ter bepaling of een klein kind in aanmerking komt voor GH-therapie.

Het groeipatroon tot in de volwassenheid van prematuur geboren kinderen is te zien in hoofdstuk 3. Drie groepen werden onderscheiden: SGA-kinderen, appropriate-for-gestational-age- (AGA-) kinderen die te licht en/of te klein waren op de leeftijd van 3 maanden post-terme (genaamd preterm-growth-restraint, PGR) en AGA-kinderen met normale proporties op 3 maanden (non-PGR). Wij vonden dat groei en eindlengte gelijk waren na SGA en AGA PGR.

De eindlengte in deze groepen was -1,1 tot -1,2 SDS, terwijl dit in non-PGR kinderen -0,4 SDS was. De groepen verschilden niet in target height SDS. Kinderen na AGA PGR die nog steeds te klein waren op de leeftijd van 5 jaar hadden een identiek risico, ongeveer 90%, als SGA-kinderen om kleine volwassenen te worden.

Hoofdstuk 4 laat zien dat prematuur geboren volwassen een taille-omtrek en een taille/heup-ratio hadden die veel groter was dan de normpopulatie. Dit gold met name voor de vrouwen. Het geboortegewicht was positief geassocieerd met de BMI SDS en de vetvrije massa op 19-jarige leeftijd. Zowel gewichtstoename tussen geboorte en de leeftijd van 3 maanden, en tussen 3 maanden en 1 jaar waren positief geassocieerd met de BMI SDS, de absolute en relatieve vetmassa, en de taille-omtrek SDS op jongvolwassen leeftijd.

In hoofdstuk 5 bestudeerden wij de effecten van geboortegewicht en vroege postnatale gewichtstoename op het lipidenspectrum en de CIMT op jongvolwassen leeftijd. Vroege groei was niet gerelateerd aan deze uitkomsten. In plaats daarvan werden sterke associaties tussen de huidige lichaamssamenstelling en het lipidenspectrum gevonden, alsmede tussen lipidenspectrum en CIMT.

De effecten van vroege groei op de parameters van insulineresistentie op 19-jarige leeftijd staan beschreven in hoofdstuk 6. Vroeggeboren volwassen waren relatief insulineresistent. Insulineresistentie werd gemeten door middel van insuline en C-peptide spiegels in nuchtere toestand alsmede met de homeostatic model assessment for insulin resistance index (HOMA-IR). De HOMA-IR wordt als volgt berekend: nuchter glucose x insuline / 22,5. Deze waarde benadert de 1 in jonge, niet-obese personen als glucose gemeten wordt in mmol/l en insuline in mU/l. Snelle gewichtstoename tussen geboorte en 3 maanden was zwak geassocieerd met hogere insulinewaarden maar niet met de andere parameters van insulineresistentie. De volwassen vetmassa was positief geassocieerd met alle parameters van insulineresistentie. Het effect van de absolute en relatieve vetmassa op deze parameters was afhankelijk van hun interactie met geboortegewicht SDS.

Een hoge prevalentie van hypertensie, i.e. een bloeddruk >140/90 mmHg volgens de Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure VII criteria, werd gevonden onder vroeggeboren volwassen, wat te zien is in hoofdstuk 7. Geboortegewicht en -lengte en postnatale groei waren niet gerelateerd aan de bloeddruk of hypertensie.

Behandeling van moeders met een dreigende vroeggeboorte met corticosteroïden ter reductie van de neonatale mortaliteit is een effectieve therapie gebleken. In proefdierstudies is de toediening van corticosteroïden echter geassocieerd met levenslange hyperglycaemie en hypertensie, en verstoorde nefrogenese in de pups. De resultaten van vergelijkbare studies in prematuur geboren personen zijn erg tegenstrijdig door een aantal belangrijke methodologische verschillen. Bovendien is de nierfunctie na maternale corticosteroïdtoediening nooit gemeten. In hoofdstuk 8 bestudeerden wij het effect van 24 mg betamethason op het meta-

bole profiel, inclusief nierfunctie, op het nageslacht. Behoudens een effect op de glomerulaire filtratie (GFR) in de vrouwen, waren er geen nadelige lange-termijn effecten van betamethason in de deelnemers op jongvolwassen leeftijd. In 19-jaar-oude vrouwen was de GFR lager na expositie aan betamethason: -7,4 (95% BI: -13,3 tot -1,5) ml/min per 1,73 m^2. Hoewel dit verschil niet klinisch relevant was op jongvolwassen leeftijd, zou het een verhoogd risico op chronisch nierfalen in prematuur geborenen vrouwen kunnen voorspellen die antenataal aan betamethason blootgesteld waren.

Een eerdere studie in een kleine groep jongvolwassenen heeft gesuggereerd dat cortisol in serum verhoogd is jaren na premature geboorte. In hoofdstuk 9 werd het effect van de R23K- en N363S-polymorphismen in het glucocorticoïdreceptor gen, geassocieerd met gevoeligheid voor cortisol, op de groei en het metabole profiel onderzocht. Dragers van de 23K-variant hadden lagere insulinewaarden en een lagere HOMA-IR dan niet-dragers. Daarnaast vertoonden deze dragers volledige inhaalgroei tussen de leeftijd van 3 maanden en 1 jaar en was de eindlengte gelijk aan het populatiegemiddelde. Het N363S-polymorphisme was aan geen enkele uitkomstmaat gerelateerd. Dus, dragers van de 23K-variant zijn, ten minste voor een deel, beschermd tegen postnataal groeifalen en insulineresistentie na premature geboorte.

Hoofdstuk 10 is een meta-analyse van publicaties over de associatie tussen geboortegewicht en basaal-cortisol. Een zoektocht in de literatuur verschafte 10 artikelen (11 populaties) met de gegevens van 2.301 proefpersonen. Verschillen tussen populaties en gebruikte methoden bemoeilijkte de vergelijkbaarheid van de studies. Na het poolen van de data vonden we een statistisch significante associatie tussen geboortegewicht en circulerend cortisol: een 1 kg lager geboortegewicht was geassocieerd met een 25,3 nmol/l (95% BI: 5,9 tot 44,8) hogere cortisolwaarde. Deze bevindingen suggereren dat er enig bewijs is voor een mogelijke rol van de hypothalamus-hypofyse-bijnier as in de epidemiologische associatie tussen geboortegewicht en cardiovasculair lijden.

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About the author

The author of this thesis was born in Leiden on the 12th of June, 1973. From 1986 to 1992 he attended secondary school (Christelijk Lyceum Dr. W.A. Visser 't Hooft) in Leiden, where he passed VWO-B level. In September 1992 he commenced the study Biomedical Sciences at the University of Leiden. Two years later he started his medical training at the same university. Both degrees were obtained in 1998. After finishing his clinical years (cum laude) in August 2000, the preparation for the work presented in this thesis started. His interest in "fetal origins" studies was acquired during a student fellowship at the Endocrine Unit of the Department of Medical Sciences of the Western General Hospital in Edinburgh (UK) from September 1997 till April 1998 (supervisor: Prof.dr. B.R. Walker). From February till December 2001 he worked as a resident at the Department of Paediatrics of 't Lange Land Ziekenhuis in Zoetermeer. During that year he obtained a grant from the Netherlands Organization for Scientific Research (NWO/AGIKO-stipendium), enabling him to combine the research resulting in this thesis with his training in paediatrics at the Leiden University Medical Center (head: Prof.dr. J.M. Wit) and at the Reinier de Graaf Gasthuis in Delft (head: Dr. N. van der Lely). This NWO/AGIKO-stipendium was started in February 2002.

The author is married with Nathalie Finken-Vorwald. They have 2 children: Marit (3) and Thijs (1). Both were born appropriate-for-gestational-age after full-term pregnancies.

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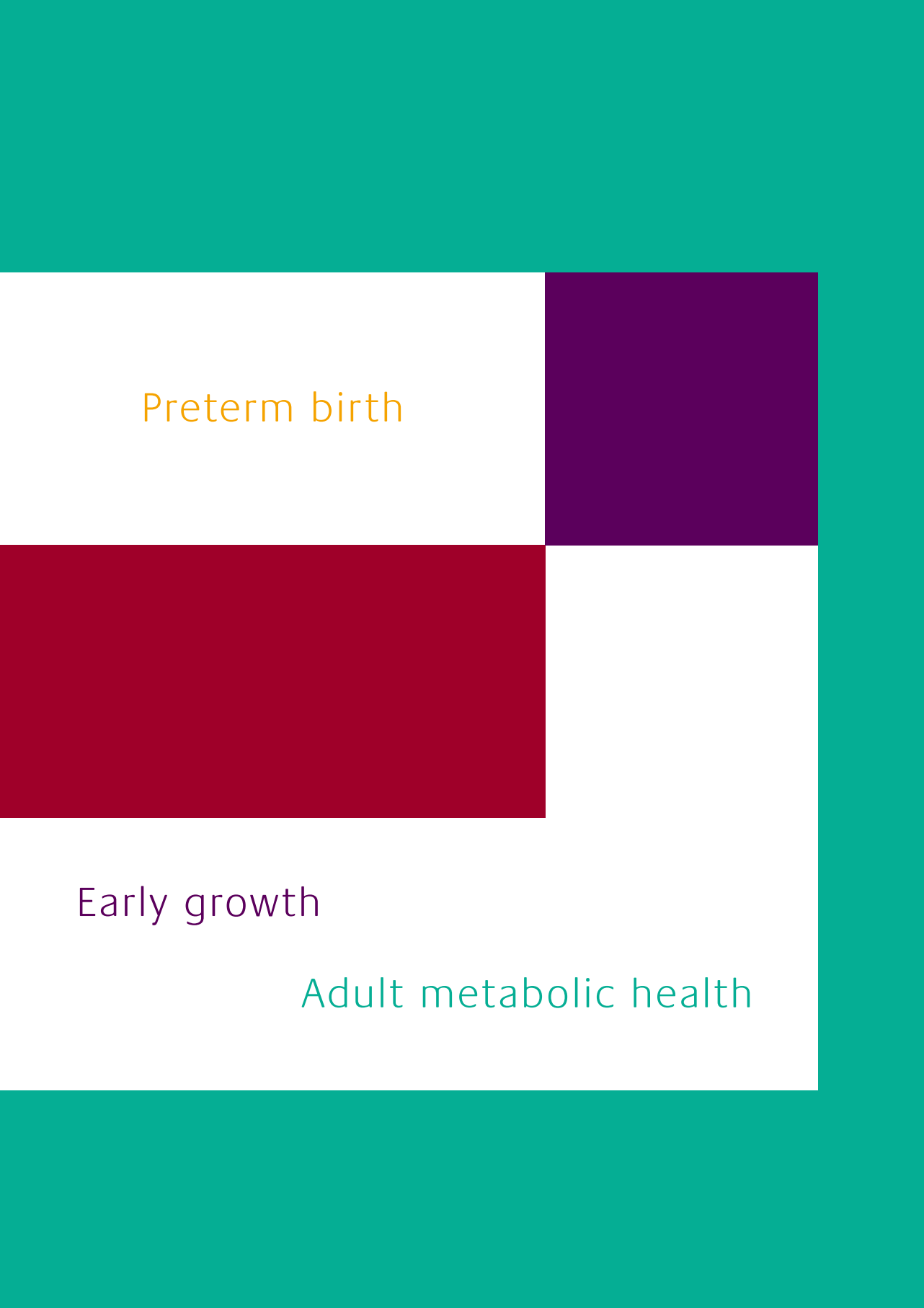
Preterm birth
Early growth
Adult metabolic health