Early Determinants of Blood Pressure and Renal Function:

Follow-up of very preterm born individuals until young adulcy

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Early Determinants of Blood Pressure and Renal Function

Follow-up of very preterm born individuals until young adulcy

Vroege determinanten van bloeddruk en nierfunctie

Follow-up van zeer prematuur geborenen tot in jong volwassenheid

Proefschrift

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As the variability of each species
is an independent property,
and will be taken advantage of
by natural selection,
only so far as it profits the individual
in its complex struggle for life,
so the degree of modification in different
species will be no uniform quantity.

Charles Darwin (1809-1882)
The origins of species, 1859

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Chapter 1

General Introduction, Objectives and Outline of this thesis

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GENERAL INTRODUCTION

With increasing morbidity and mortality of cardiovascular disease and end stage renal disease, risk factors contributing to these diseases are frequently studied in the last years.[1;2] Besides some well known factors (e.g., smoking, obesity, hypertension and type II diabetes) also early determinants, such as intrauterine undernutrition, rapid postnatal growth, and prematurity are suggested to increase the susceptibility to these diseases. The associations between early determinants and increased susceptibility to adult diseases is also known as the 'developmental origins of adult health and disease' hypothesis.[3-5]

In this thesis several aspects of the 'developmental origins of adult health and disease' hypothesis are discussed, focussed on renal development, adult renal function and hypertension. After this general introduction the objectives and outline of this thesis are described.

'Developmental origins of adult health and disease' hypothesis

In 1986 a correlation between birth weight and ischaemic heart disease in adults in England and Wales was suggested.[6] In this study (post)neonatal mortality in the period of 1921 – 1925 was positively related to ischaemic heart disease in the same regions in the period of 1968-1978. Also other diseases (bronchitis, stomach cancer and rheumatic heart disease) were related to infant mortality, suggesting that poor living conditions, including poor nutrition, in early life is an important determinant in development of disease on the short and long run. Furthermore, weight at the age of 1 year was negatively related to cardiovascular mortality at adult age.[7] Therefore, it was suggested that processes linked to growth and acting in prenatal or early postnatal life strongly influence the risk of ischaemic heart disease. Associations between fetal growth, (early) postnatal growth and adult weight and body composition have been demonstrated.[8-10] Many studies concerning this 'developmental origins of adult health and diseases' hypothesis (formerly known as 'fetal origins of adult diseases' or 'Barker' hypothesis) are published in the years thereafter.[11-14] A relation between prenatal or postnatal circumstances and the development of adult cardiovascular disease and hypertension, insulin resistance and diabetes mellitus type II[4;15;16], obesity and the metabolic syndrome[8;9], renal disease[17;18], and the hypothalamo-pituitary adrenal axis[19;20] has been documented in both animal experiments and in human observational studies.

Also the effect of accelerated postnatal growth in weight and length are suggested to increase the risk for developing hypertension and type II diabetes in later life, especially in low birth weight individuals.

Thriphty phenotype hypothesis, programming and developmental plasticity.

Redistribution of nutrients in favour of the development of the brain is an important consequence to survive insufficient fetal nutrition. Maternal malnutrition (insufficient intake or unbalanced nutrients) and placenta insufficiency are the most common causes of fetal malnutrition and intrauterine growth retardation (IUGR).[21] Fetal nutrient redistribution leads to changes in cardiac output, hormone production, and sensitivity to hormones.[22] These changes are thought to affect development of lungs, pancreas and kidneys. Adaptation to the 'low nutrient' situation may lead to an increased susceptibility for malfunction of these specific organs after birth when this 'low nutrient' situation is discontinued.[23] Terms as 'thriphty phenotype' hypothesis, 'developmental plasticity', 'programming', all refer to this ability of adaptation.[24]

It is likely that organs and organ system are affected most when undernourishment is present in the period of their 'critical window' of development. In the Dutch famine studies it was demonstrated that undernutrition in early gestation led to an increase in coronary heart disease, atherogenic lipid and thrombotic blood profile, and obesity. [25] Mid-gestation undernutrition increased the obstructive airway disease and microalbuminuria prevalence, and undernutrition during late gestation mainly increased glucose tolerance.[25]

Besides nutrition, other environmental factors, such as hypoxia, stress, and vitamin A deficiency, and variation in genes and changes in genetic expression have been linked to early development and adult diseases susceptibility.[26-29] Also maternal factors, such as age, maternal diabetes, hypertension during pregnancy, maternal smoking during pregnancy, and maternal genetic factors may influence the metabolic pathway in the offspring.[17;30-32] Future studies are warranted to elucidate the role of these factors.

Pathophysiological mechanisms

Several pathways have been proposed as possible explanations for the occurrence of adult diseases after IUGR, and reviewed by McMillen et al.[33] Pancreatic underdevelopment with β-cell dysfunction, upregulation and downregulation of hepatic glucose-related cell receptors, and development and function of the adipocyte and smooth muscle cells all seem to contribute to the development of glucose tolerance, insulin resistance and type II diabetes in IUGR individuals.[33] Development of

cardiovascular disease and hypertension may be explained by alterations in the renin-angiotensin-system, (vascular) endothelial dysfunction, and impaired renal development with a nephron deficit.[33] Also the occurrence of (end-stage) renal disease during life is linked to this impaired (fetal) renal development after IUGR.[34]

Renal involvement

Nephron endowment

A relation between a reduced nephron number and development of essential hypertension has been long recognized, also known as the 'hyperfiltration theory'.[35] This theory enables to understand the possible biological mechanism in the hypothesis that renal diseases at adult age is associated with IUGR (Figure 1).[36-38] Prenatal malnutrition may lead to IUGR and impairment of renal development including deficit in nephron numbers, which causes a decreased filtration surface area. Renal hemodynamic alterations, like (single-nephron) hyperfiltration, are needed to optimalize the glomerular filtration in order to maintain normal renal function. [36-38] This phenomenon is accompanied by glomerular and systemic hypertension[36-38], as also demonstrated in individuals with unilateral agenesis and ablative surgery in kidney donors.[39;40] Persistent glomerular hypertension causes damage of nephrons leading to acquired glomerular sclerosis.[41] Nephron number decreases followed by a decrease in filtration surface area (vicious circle) eventually resulting in (end stage) renal disease.[36-38]

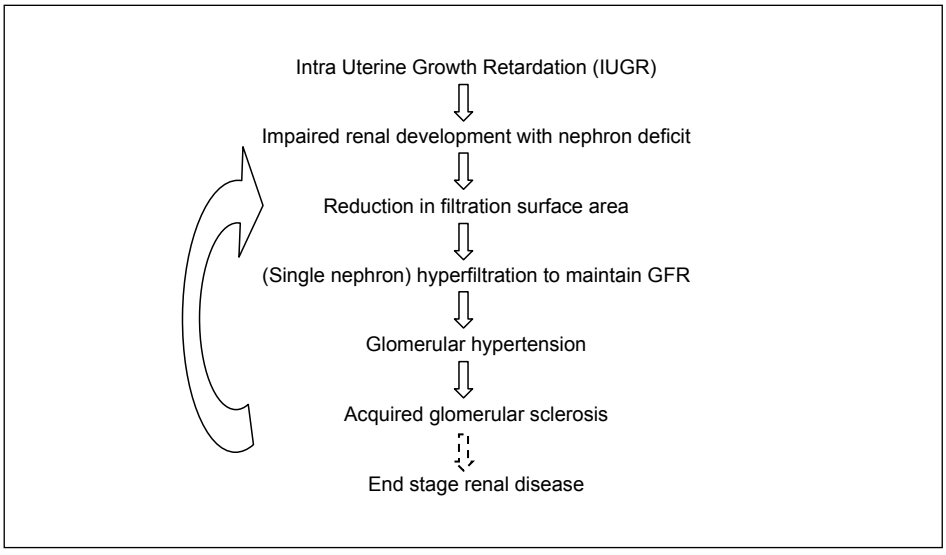

Figure 1: Brenners Hyperfiltration theory. [38]

Recently, data are published showing a strong relation between birth weight and renal size, nephron number, albuminuria and systolic blood pressure in Aboriginal communities.[34] In these communities, diseases like type II diabetes, cardiovascular diseases and renal diseases take epidemic proportions.[42;43] In 668 aboriginals between 4 and 72 years, those individuals with the lowest birth weights had smallest kidneys (measured by ultrasound), and the individuals with the smallest kidney size had the highest blood pressure and the highest rates of albuminuria (ACR ≥ 34 mg/mmol).[34] In the children (n=210 < 15 years), with a 1 cm increase in mean kidney length systolic blood pressure decreased with 3.2 mmHg.[34] Another study in 56 deceased African Americans and Caucasians the kidneys were microscopically investigated.[44] Nephron number, measured by stereologic estimation, was related to birth weight. Nephron number also was inversely related to glomerular volume, suggesting that glomerulomegaly is a marker of an increased risk of groups of patients or populations with progressive renal disease.[45;46] These findings support Brenners 'hyperfiltration theory' indicating that birth weight is a risk factor for the development of progressive renal disease. [36]

Renin-Angiotensin-System

The renin-angiotensin-system (RAS) is important in the regulation of systemic and glomerular blood pressure. A decreased renal blood flow leads to an increase in the production of renin by juxta-glomerular apparatus, and increased blood pressure in two ways. First, renin is converted into angiotensin I (AT I), which is converted into angiotensin II (AT II) by angiotensin-converted enzyme (ACE). AT II stimulates α-receptors causing vasoconstriction in small vessels leading to an increase in systemic blood pressure. Second, AT II stimulates aldosterone hormone production in the adrenal glands that stimulates sodium reabsorption and water retention in the distal tubulus leading to an increase of circulating volume and systemic blood pressure.[47]

In addition to the role of blood pressure regulation, the RAS is also thought to be an important factor in the fetal renal development.[48] Renal structural abnormalities, such as papillary atrophy and pelvic dilatation, are shown in animals in whom RAS was blocked during renal development.[49] Furthermore, nephrovascular development was impaired in rats after $ATII_1$ receptor blockade, with extensive vascular abnormalities of the afferent arteriolae and cortical radial arteries.[50] In humans, oligohydramnios and prolonged neonatal anuria have been reported after fetal exposure to ACE inhibitors.[51;52]

Besides these structural changes, increased blood pressure, decreased renal function, and altered renin concentrations were observed after birth in rats after RAS suppression during nephrogenesis.[53-55] However, literature on this topic is fragmented. Experimental studies have demonstrated RAS suppression in the offspring

of protein restricted pregnant rats.[56] This was also associated with a inhibition of nephrogenesis and development of the ascending limb of Henle.[56] Also, increased $ATII_1$ receptor expression was shown in undernutritioned fetal rats.[57] This may be a direct effect of protein restriction or a response to a decreased ATII concentration.[57]

In humans, Konje studied the active renin levels and AT I levels in the umbilical vein in preterms and IUGR human fetuses.[58] AT I levels were increased in IUGR infants born preterm. Hypoxia, increased sympatic nerve activity and catecholamin production (all present in IUGR fetuses), but also proliferation of juxta-glomerular cells (and thus renin producing cells) were suggested to be involved. Martyn showed a decreased inactive renine concentration in human adults exposed to malnutrition and IUGR.[59] The differences in study design, exposure and outcome variables (e.g., (in)active renin, ATI, ATII, ATII receptor) make studies hard to compare. Also the association between fetal alterations in RAS activity and adult blood pressure or renal function are not convincing. Large field of research unclear biological pathway. Further investigations are needed to elucidate the role of RAS in fetal kidney development and the association with adult hypertension and renal function loss.

Gestational age and the 'developmental origins of adult health and disease' hypothesis

In humans, rapid development of important functional cell structures in the lungs, pancreas and kidneys takes place until the last few weeks of gestation and preterm birth may affect final development.[60-62] For example, nephrogenesis continues the gestational age of 36 weeks.[62] It has been suggested that premature birth impairs final development of nephrons (after birth).[63;64] This was observed in 56 deceased premature infants with extremely low birth weight (birth weight ≤ 1000 gram of which 25% SGA) and 10 deceased full term born controls with normal birth weight.[63] Nephron number was highly correlated to gestational age and active glomerulogenesis was ceased after 40 days postnatal. Therefore, a reduced nephron number after preterm birth persists throughout life and may affect long-term renal function and blood pressure.

Several studies support the hypothesis that preterm birth may have an additional effect to low birth weight in the increased risk for diseases in later life.[5;65-67] A large Swedish study showed an inverse association between GA, ranging from 35 - 44 weeks, and systolic BP (SBP) in 165,136 Swedish men.[5] This correlation may be stronger in the lower range of gestation (GA 30-38 weeks).[65] Similar results were found in young adult women between 23 and 30 years of age.[66;67] Systolic blood pressure and the number of hypertensive blood pressure readings were only increased in the preterm born individuals with appropriate birth weight, and not in full term born controls (either IUGR or with appropriate birth weight).

Moreover, it was suggested that growth and development during the third trimester after conception of the preterm infant was more important in development of cardiovascular risk factors than intrauterine growth retardation alone.[68] Adult blood pressure and fasting glucose levels were higher in individuals who were born very premature compared with normal birth weight controls. No difference between the individuals who were born very premature with IUGR or appropriate birth weight was present. Other studies comparing individuals who were born very premature with either low or appropriate birth weight are scarce.[69]

In this thesis we describe the long term follow-up of very premature born individuals, both small and appropriate for gestational age and compared the results with full term born controls with appropriate birth weight.

OBJECTIVES OF THIS THESIS

The main objectives of the studies described in this thesis were to evaluate the effect of premature birth and of IUGR within very preterm born individuals on blood pressure and renal function at young adult age. Two studies were conducted to answer the following research questions:

1. Is IUGR a predisposing factor for increased blood pressure at young adult age within individuals who were born very premature?
2. Is blood pressure increased at young adult age in individuals who were born very premature and IUGR compared with individuals who were born very premature with appropriate birth weight, and with full term born controls?
3. Is IUGR a predisposing factor for decreased renal function at young adult age within individuals who were born very premature?
4. Are renal function and renal functional reserve capacity (RFRC) impaired in individuals who were born very premature and IUGR compared with individuals who were born very premature with appropriate birth weight, and with full term born controls at young adult age?
5. Do individuals who were born IUGR and / or prematurely have smaller (relative) kidney size at young adult age compared with full term born controls?
6. Is there an association between renal size, renal function, renal functional reserve capacity, and blood pressure in very preterm born individuals and in controls? or Do subjects with an increased blood pressure also have a decreased renal function and a decreased renal size?

Blood pressure and renal function were obtained from the individuals who participated in the follow-up visit at the age of 19 years of the 1983 very preterm birth cohort (Project On Prematures and Small for gestational age infants: POPS) between April 2002 and May 2003. Additionally, we examined renal function, renal functional reserve capacity, renal size, and 24-hour blood pressure in 52 individuals who were born very premature and in 30 controls in the ErasmusMC-Sophia Children's Hospital, University Medical Center Rotterdam, The Netherlands between October 16th 2003 and November 18th 2004.

OUTLINE OF THIS THESIS

In the first chapter of this thesis an overview of the literature is given, followed by the main objectives and outline of this thesis. Chapter 2 describes a statistical approach in the analyses of the 'developmental origins of adult health and disease' hypothesis. The study methods of the *POPS 19 study* are described in Chapter 3.1 followed by the results on blood pressure (Chapter 3.2) and renal function (Chapter 3.3). Chapter 4.1 describes the methods of the *POPS Nephrology study*. The results of this study on blood pressure (Chapter 4.2), renal function (Chapter 4.3) and renal ultrasound (Chapter 4.4) are discussed in the following chapters. The general discussion (Chapter 5) answers the main research questions of this thesis, as described in Chapter 1.

Chapter 2

Analysing the 'fetal origins of adult diseases hypothesis' using a regression model with 'unexplained residuals'

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JP Vandenbroucke, JC van Houwelingen.

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ABSTRACT

Background and objective: A continued controversy exists whether the assessment of the influence of low birth weight on adult blood pressure necessitates adjustment for adult weight in the analysis on the fetal origins of adult diseases hypothesis. Here we first explain the difficulty in understanding an adjusted multivariate regression model, and second to propose another way of writing the regression model to make the interpretation of the separate influence of birth weight and changes in weight later in life more straightforward.
Methods: We used a multivariate regression model containing birth weight (SDS), and residual adult weight (SDS) to explore the effect on blood pressure (or any other outcome) separately. Residual adult weight was calculated as the difference between actual adult weight and the expected adult weight (SDS) given on a certain birth weight (SDS).
Results: The coefficients of birth weight and residual adult weight show directly the effect on the analysed outcome variable.
Conclusions: We prefer to use this regression model with unexplained residuals when the adjusted variable is in the causal pathway in the analyses of data referring to the fetal origins of adult diseases hypothesis.

INTRODUCTION

In the literature on the fetal origins hypothesis, a continued controversy exists whether the assessment of the influence of low birth weight on adult blood pressure necessitates adjustment for adult weight [70;71]. The controversy was fueled by the meta-analysis of Huxley et al. who described little or no relation between birth weight and adult blood pressure if unadjusted for adult weight, and implied that such adjustment might even be misleading [72]. The effect of adding adult weight as a variable in the regression of blood pressure on weight at birth is intricate: a review by Lucas et al. suggested that such a regression model should in fact be interpreted as the influence of a change in weight between birth and adulthood – and no longer as the influence of birth weight [73]. Nonetheless, the interpretation of data by this concept remains confusing.

Our objective here is first to explain the difficulty in understanding the adjusted regression for the general reader, and then to propose another way of writing the regression model to make the interpretation of the separate influence of birth weight and changes in weight later in life more straightforward. We will explain the model not only conceptually and algebraically, but also by an example on data from an ongoing study on the effect of birth weight on blood pressure. Validation of the model in future analysis is warranted.

The adjusted regression analysis

Originally the association between birth weight and adult blood pressure was mainly analysed without adjustments for additional variables [74]. Later, it was shown that subjects born with low birth weight tended to gain more weight compared with subjects born with a normal birth weight. Weight gain alone was also associated with an increased risk for high blood pressure. Therefore, adult weight was seen as a potential confounder in the analysis, and adjustment for it became more common [12]. Some articles, however, found a significant association between birth weight and adult diseases only after adjustment for adult weight [75]. Therefore, the need for a multivariate regression model incorporating the effects of both birth weight and adult weight seemed to be the most promising statistical approach. Still, the interpretation of what was achieved by this adjustment remained unclear.

Lucas et al. outlined the consequences of adjustment for adult weight (or length) in a multivariate regression analysis [73]. They proposed using four regression models to analyse the data (Table 1), and stated that in the adjusted models the early and later size of the subjects can no longer be interpreted as stand- alone variables:

adjusting early size for later size is a measure of change in size between the earlier and later measurement. In their terminology the *early model* describes the relation between early size (i.e., birth weight, or bw) and outcome ($Y = \alpha_1 + \beta_1 X_{bw}$). In the *late model*, the relation between later size (i.e., adult weight, or aw) and outcome is studied ($Y = \alpha_2 + \gamma_2 X_{aw}$). The *combined model* (adding later size to the early model) can be interpreted as describing the relation between change in size and outcome ($Y = \alpha_3 + \beta_3 X_{bw} + \gamma_3 X_{aw}$), as argued by Lucas et al. (Table 1). Adding the interaction term of early and later size to the model yields the *interaction model*, allowing the exploration of whether early size affects the relation between later size and outcome ($Y = \alpha_4 + \beta_4 X_{bw} + \gamma_4 X_{aw} + \delta_4 X_{bw} X_{aw}$).[73] Note, however, that the changing coefficients (in size and direction) in the combined and interaction models compared to the early model result in a complicated interpretation. Indeed, the effect of later size is co-determined by the effect of early size on outcome, because adult weight is determined in part by birth weight, which influences the coefficients in the combined model. This also implicitly assumes a quadratic relation between birth weight and outcome in the interaction model, at least under the assumption that birth weight and adult weight are linearly related (Table 1).

Table 1: Interpretation of multivariate regression model by Lucas et al.[73].

Early model: regression analysis of early weight (*ew)* to outcome measure
$Y = \alpha_1 + \beta_1 X_{bw}$
Late model: regression analysis of adult weight (*aw)* to outcome measure
$Y = \alpha_2 + \gamma_2 X_{aw}$
Combined model: adding later size to early model
$Y = \alpha_3 + \beta_3 X_{bw} + \gamma_3 X_{aw}$
Interaction model: adding the interaction of early and adult size to the combined model.
$Y = \alpha_4 + \beta_4 X_{bw} + \gamma_4 X_{aw} + \delta_4 (X_{bw} X_{aw})$
Interaction model with subtraction of the means:
$Y = \alpha_4 + \beta_4 X_{bw} + \gamma_4 X_{aw} + \delta_4 ((X_{bw} - \bar{X}_{bw})(X_{aw} - \bar{X}_{aw}))$

X_{bw} = birth weight; X_{aw} = adult weight; $X_{bw} X_{aw}$ = interaction of birth weight and adult weight; Y = expected outcome; α = intercept; β, γ, δ = coefficient.

Which analysis meets the researcher's concerns?

Whether later size (e.g., adult weight) is a confounder in the analysis of early size (e.g., birth weight) and adult diseases, such as hypertension, or is rather a factor in the causal pathway is an ongoing debate in the literature. Adjustment for adult weight might not be justified after all [70-72]. Whatever the causal explanation, birth weight is positively correlated with adult weight and adult weight is correlated with adult blood pressure; therefore, we do first of all expect that any positive relation between birth weight and adult blood pressure will be attenuated upon adding adult weight to the model (the coefficient of birth weight will become closer to

zero). Next, according to Lucas, it might be those who grew more than expected (i.e., attained greater adult weight for a given birth weight) who would develop the higher blood pressures. This would reverse the already attenuated relation with birth weight into a negative relation.

As researchers, we remain interested in the separate contribution of birth weight (reflecting pre-birth influences) and change in weight from birth to adulthood (reflecting early life influences). Thus, we want to have an estimate of both. We want first an estimate of the effect of birth weight alone, and second, what we really want to know is the effect of someone growing more in weight than would be expected from a given birth weight. In a statistical analysis this can be accomplished in a single model by first calculating the expected adult weight, or eaw, based on birth weight ($X_{eaw} = \alpha_0 + \beta_0 X_{bw}$), and then subtract expected adult weight from actual adult weight – which is in effect the calculation of a residual ($X_{res} = X_{aw} - X_{eaw}$) (Table 2).

Table 2: Interpretation of unexplained residual regression model

Early model: regression analysis of early weight to outcome measure
$Y = \alpha_1 + \beta_1 X_{bw}$
Late 'unexplained residual' model: regression analysis of residual of expected adult weight to outcome measure. First expected adult weight *(eaw)* is calculated: X_{eaw} = expected adult weight, based on birth weight ($\alpha_0 + \beta_0 X_{bw}$). Then, the residual for expected adult weight is calculated as: $X_{res} = (X_{aw} - X_{eaw})$ leading to $Y = \alpha_2 + \gamma_2 X_{res}$
Combined 'unexplained residual' model: adding the residual of the expected adult weight to early model
$Y = \alpha_3 + \beta_3 X_{bw} + \gamma_3 X_{res}$
Interaction 'unexplained residual' model: adding the interaction the difference between birth weight and the mean birth weight and the difference between the residual and the mean residual of the expected later size to the 'combined unexplained residual model'.
$Y = \alpha_4 + \beta_4 X_{bw} + \gamma_4 X_{res} + \delta_4((X_{bw} - \bar{X}_{bw})(X_{res} - \bar{X}_{res}))$ in which $\bar{X}_{res}$ is zero.

In this model $\beta_1 = \beta_3 = \beta_4$ and $\gamma_2 = \gamma_3 = \gamma_4$.

X_{bw} = birth weight; X_{res} = residual of expected adult weight, based on birth weight; $(X_{bw} - \bar{X}_{bw})(X_{res} - \bar{X}_{res})$ = interaction of birth weight and residual of expected adult weight; Y = expected outcome; α = intercept; β, γ, δ = coefficient

Adding this residual increase in weight in a regression model of blood pressure on birth weight has three advantages. First of all it leaves the coefficient of birth weight unchanged (because the effect of birth weight on adult weight is already taken out of the residual). Second, it gives us an insight into the additional influence of growing more in weight than expected upon the adult blood pressure. Thirdly, the two variables in the regression model (birth weight and the residual increase in weight) are now independent, because the residual cannot be predicted from birth weight. Therefore, the interaction model does not assume a quadratic relation anymore. Li

et al. earlier described this model in the analyses of a Guatemalan study in which the association between prenatal and postnatal growth and adult body composition was studied [76]; however, no algebraic explanation of this model was shown.

The proposed technique is not unique to the problems of interpreting regression in the fetal origins to adult diseases hypothesis. It has been used in social sciences literature under the name of residualized gain score [77;78].

It should be noted that algebraically the combined model of Lucas et al. is the same as the combined model using unexplained residuals (Appendix A); however, the effect of birth weight and residual postnatal growth is directly shown by the coefficients of the proposed unexplained residual regression model. In both models, for the interaction model we suggest to multiply not just the two variables, but first subtract the mean of that variable. In the Lucas model this becomes $(X_{bw} - \bar{X}_{bw})(X_{aw} - \bar{X}_{aw})$; in the proposed model this becomes $(X_{bw} - \bar{X}_{bw})(X_{res} - \bar{X}_{res})$. As the mean of a residual is zero, this can be rewritten in $(X_{bw} - \bar{X}_{bw})X_{res}$.

Next to the model of Lucas et al.. other simplified models are suggested to use in the fetal origins to adult diseases hypothesis analysis to measure the effect of change in weight. When researchers think about the problem they often intuitively propose to subtract adult weight (SDS) and birth weight (SDS) as a measure of change in weight and add this to birth weight (SDS) in a multivariate regression model. However, the problem with this model is the phenomenon of regression to the mean. The relative position of subjects with low birth weight will tend to increase and that of subjects with high birth weight will tend to decrease over time. This phenomenon is not present in de unexplained residual model, because in the calculation of adult weight residuals out of birth weight we force the residuals not to be related to birth weight. The coefficient of birth weight in a linear regression model of adult weight residuals is exactly zero (with very small confidence interval and a P-value of exactly 1).

Second, it has also been suggested to use population based SD scores instead of calculating the residual of expected adult weight (SDS). However, the subjects studied in research concerning the fetal origins to adult diseases hypothesis are mostly not comparable to the general population, because of an overrepresentation of the low birth weight subjects. Subjects with low birth weight have different growing patterns. Therefore, for most studies it is not recommended to use population-based SD scores to calculate expected adult weight (SDS) and weight gain (SDS). In addition, it takes about three years after birth before an individual will track on his or hers centile, especially in low birth weight infants. If the population-based reference standards were to be used as a measure for expected adult weight (SDS), in which the mean adult weight (SDS) will be zero, low birth weight (SDS) subjects will tend to have a negative residual for adult weight, because of their suboptimal

growth. Then, the residual would not reflect the correct variable to answer our second question: what is the effect someone growing more in weight than would be expected from a given birth weight? So, calculating the residual adult weight out of birth weight should be performed with the expected adult weight from the group of subjects that are used in the study.

CONCLUSION, AND PROPOSAL

Algebraically, the combined model of Lucas et al. and our combined model with the residuals increase in weight can be rewritten in each other, except for the situation where an interaction term is entered (Appendix A). In the proposal by Lucas et al., however, one needs two separate models: first to estimate the coefficient from the early model, and then looking at the coefficient for attained weight in the combined model (without paying attention to the coefficient of birth weight in that combined model, as the latter has become meaningless). For this reason, we prefer the proposed model with residuals because it permits in a more straightforward way to estimate the effect of birth weight and the effect of additional weight gain in a single model. We also prefer to use the interaction model containing the unexplained residuals, because no quadratic relation is assumed and because in principle all coefficients show their own effect without mutual influence (see Table 2). Therefore, the interpretation of the model with the unexplained residuals is easier. An example with numerical data from an ongoing study in the Netherlands is given in Appendix B, including tables B1 and B2.

In conclusion, we prefer to use regression model with unexplained residuals when the adjusted variable is in the causal pathway in the analyses of data referring to the fetal origins of adult diseases hypothesis.

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APPENDIX A

Derivations

To rewrite the combination *unexplained residual model* in the *combined model* by Lucas et al.[73], where Y is the expected outcome; α is the intercept; β is a coefficient; X_{bw} is the birth weight; X_{aw} is the adult weight; X_{eaw} is the expected adult weight, based on early size $(\alpha_0 + \beta_0 X_{bw})$; and X_{res} is the residual of expected adult weight $(X_{aw} - X_{eaw})$

$Y = \alpha_1 + \beta_1 X_{bw} + \gamma X_{res}$ (the unexplained residual model)
$Y = \alpha_1 + \beta_1 X_{bw} + \gamma(X_{aw} - (\alpha_0 + \beta_0 X_{bw}))$
$Y = \alpha_1 + \beta_1 X_{bw} + \gamma X_{aw} - \gamma\alpha_0 - \gamma\beta_0 X_{bw}$
$Y = (\alpha_1 - \gamma\alpha_0) + (\beta_1 - \gamma\beta_0) X_{bw} + \gamma X_{aw}$
$Y = \alpha' + \beta' X_{bw} + \gamma' X_{aw}$ (Lucas model)
$\alpha' = \alpha_1 - \gamma\alpha_0$
$\beta' = \beta_1 - \gamma\beta_0$
$\gamma' = \gamma$

To add the interaction term $((X_{bw} - \bar{X}_{bw})*(X_{aw} - \bar{X}_{aw})$ into the Lucas model, first suppose that X_{aw} is exactly linearly related to X_{bw}. Then, where ε is the residual:

$$X_{aw} = \alpha_0 + \beta_0 X_{bw} + \varepsilon \text{ (in which)}$$

and

$$\bar{X}_{aw} = \alpha_0 + \beta_0 \bar{X}_{bw}$$

So,

$$(X_{aw} - \bar{X}_{aw}) = (\alpha_0 + \beta_0 X_{bw} + \varepsilon) - (\alpha_0 + \beta_0 \bar{X}_{bw})$$

which can be rewritten as

$$(X_{aw} - \bar{X}_{aw}) = \beta_0 (X_{bw} - \bar{X}_{bw}) + \varepsilon$$

Adding this to the interaction term $(X_{bw} - \bar{X}_{bw})*(X_{aw} - \bar{X}_{aw})$, the equation becomes:

$$(X_{bw} - \bar{X}_{bw})*(\beta_0 (X_{bw} - \bar{X}_{bw}) + \varepsilon)$$

This can be rewritten as

$$\beta_0 (X_{bw} - \bar{X}_{bw})^2 + (X_{bw} - \bar{X}_{bw}) * \varepsilon$$

Here, the quadratic relation between birth weight and outcome is shown.

To add the interaction term into the unexplained residual model:

$$(X_{bw} - \bar{X}_{bw})(X_{res} - \bar{X}_{res}) = (X_{bw} - \bar{X}_{bw}) X_{res}$$

In this model X_{res} (the residual of expected adult weight) is independent of X_{bw} (birth weight). All coefficients show the (unadjusted) effect of the variable on outcome variable.

APPENDIX B

Example of regression analysis according to Lucas and the *unexplained residual model* [73] (Tables B1 and B2): In a prospective study the systolic blood pressure at adult age was measured. Birth weight standard deviation scores (BW-sds) and adult weight standard deviation scores (AW-sds) were known.

Table B1: Estimated coefficients in our example when Lucas model is used, two types of interaction used.

Model Lucas	α	β	γ	δ
		X_{bw}	X_{aw}	$X_{bw}X_{aw}$
Early	122.943 (α_1)	0.361 (β_1)		
Late	123.908 (α_2)		2.069 (γ_2)	
Combined	123.743 (α_3)	- 0.0928 (β_3)	2.096 (γ_3)	
Interaction	123.771 (α_4)	- 0.0078 (β_4)	2.231 (γ_4)	0.120 (δ4)
		X_{bw}	X_{aw}	$(X_{bw} - \bar{X}_{bw})^*$ $(X_{aw} - \bar{X}_{aw})$
Interaction with subtracted means	123.710 (α_4)	- 0.0766 (β_4)	2.125 (γ_4)	0.120 (δ4)

α = intercept; β, γ, δ = coefficient; X_{bw} = birth weight (SDS); X_{aw} = adult weight (SDS)

Table B2: Estimated coefficients in our example when 'unexplained residual model' is used.

Model unexplained residuals	α	β	γ	δ
		X_{bw}	X_{res}	$(X_{bw} - \bar{X}_{bw})_{Xres}$
Early	122.943 (α_1)	0.361 (β_1)		
Late	123.623 (α_2)		2.096 (γ_2)	
Combined	123.943 (α_3)	0.361 (β_3)	2.096 (γ_3)	
Interaction	123.943 (α_4)	0.361 (β_4)	2.121 (γ_4)	0.102 (δ4)

α = intercept; β = coefficient; X_{bw} = birth weight (SDS); X_{res} = residual of expected adult weight (SDS); MeanX_{res} equals zero in interaction term $(X_{bw} - \bar{X}_{bw})(X_{res} - \bar{X}_{res})$.
Expected adult weight (X_{eaw}) = - 0.382 + 0.216* $X_{bw.}$ Residual of adult weight (X_{res}) = X_{aw} - X_{eaw}

In Table B1, the change in estimated coefficients is shown in both the combined as the interaction model, both with and without the subtractions of means, when the model of Lucas et al. is used.[73] In the early model birth weight (SDS) is related to blood pressure with a coefficient of 0.361. When adult weight (SDS) is added to the model the coefficient for birth weight (SDS) changed into a negative one (-0.0928). This is a result of the relation between birth weigth (SDS) and adult weight (SDS). This change in the estimated coefficient is confusing for many authors; which coefficient is giving information about the relation between birth weight (SDS) and blood pressure?

In the combined 'unexplained residuals' model these estimated coefficients do not change (Table B2) when adult weight (SDS) is added to the model. The coefficient for birth weight and residual weight gain shift slightly in the interaction model in comparison with the combined 'weight residual model': probably this is due to non-exact-linear correlation between birth weight and weight gain.

The δ4 coefficient does not change much in our example. The reason is that X_{bw} is not related to systolic blood pressure. Therefore, the δ4 coefficient in the Lucas model is comparable to the δ4 coefficient in our model. When X_{bw} would be quadratically related to blood pressure, the δ4 coefficient would differ much in both models.

Chapter 3

POPS 19 study: Study design, blood pressure and renal function

Chapter 3.1

Study design POPS 19 study

INTRODUCTION

Since the introduction of Neonatal Intensive Care Units in the sixties the chance of surviving very premature birth and (very) low birth weight has improved dramatically.[79-81] Use of high quality techniques and medication not only led to a decrease in mortality rates, but also to an increased concern on long-term outcome.[82] General health, neuromotoric and cognitive development, and behaviour have been shown impaired after very preterm or low birth weight birth.[83-85]

PROJECT ON PREMATURES AND SMALL FOR GESTATIONAL AGE INFANTS (POPS)

A birth cohort of very preterm born infants (gestational age <32 weeks) and very low birth weight infants (birth weight < 1500 g) was recruited in 1983 in the Netherlands to obtain obstetric, perinatal, and neonatal parameters and evaluate survival and morbidity.[86] Of all eligible neonates, 94% were included in this follow-up study (*N*=1338), which equals 0.7% of all births in the Netherlands in 1983 (Figure 1). During follow-up, almost 30% of the included individuals died, mainly during the first year of life. Follow-up was assessed at 3, 6, 12, and 24 months corrected age and at the age of 5 years to obtain information on general health, growth and development of these infants. Questionnaires on health, disabilities, school performance and quality of life were completed at the ages of 9, 10, and 14 years. [83] The majority (90%) of the individuals have survived without severe disabilities at school age, but many meet serious difficulties in everyday life.

STUDY DESIGN POPS 19 STUDY

At the age of 19 years a new follow-up study of the POPS cohort was conducted to obtain information on general health, psychological and social aspects (POPS 19 study). General health included parameters of Syndrome X (blood pressure measurement, anthropometric measurements, ultrasound of carotic arteries to estimate Intima Media Thickness and laboratory diagnostic examination of blood and urine samples), neuromotoric examination, audiologic test, cognition test, pain coping test (Figure 2). Psychosocial parameters were obtained using several questionnaires, filled out by the participants and their parents (eg, Young Adult Self Report, Young Adult Behaviour CheckList, Health Utility Index, London Handicap Scale).

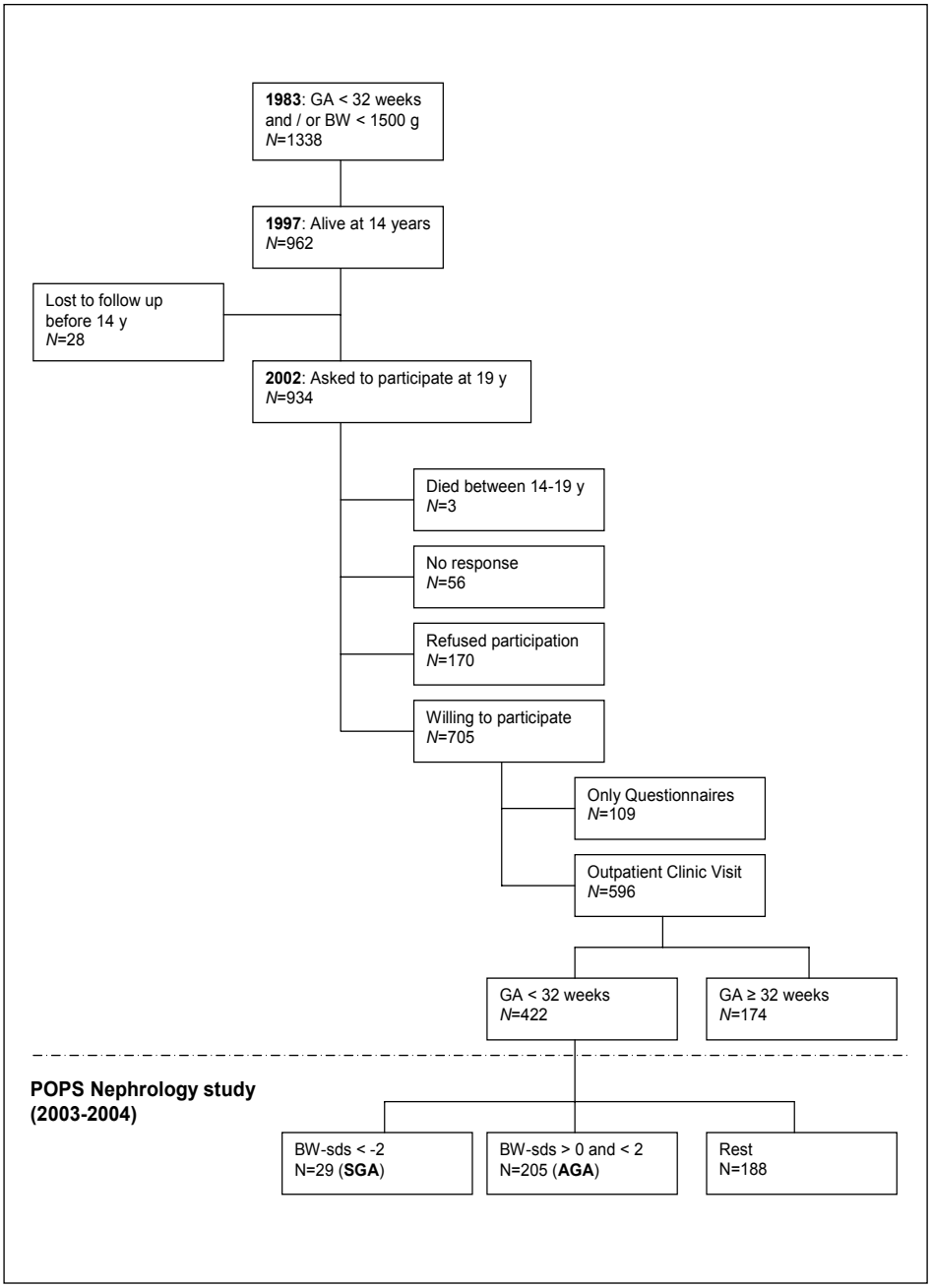

Figure 1: Flow chart inclusion of participants of the POPS 19 study (2002-2003)

All individuals were recruited by mailings and phonecalls performed by TNO Quality of Life, Leiden, The Netherlands. All participants were invited to visit one of ten outpatient clinics for the follow-up visit between April 2002 and May 2003. At the age of 14 years, 962 individuals were alive, and 28 individuals were lost to follow up, leading to 934 individuals eligible for inclusion at the age of 19 years. (Figure 1) Eventually, 596 individuals participated in the POPS 19 follow-up study.

All ten participating outpatient clinics were involved in a specific part of the POPS 19 study protocol. Parameters on Syndrome X were analysed in collaboration between the Erasmus MC – Sophia Children's Hospital, University Medical Center Rotterdam, Department of Pediatric Nephrology and the Leiden University Medical Center, Department of Clinical Epidemiology and Department of Pediatrics.

Day 1	Morning urine collection
	10.00 p.m. start 24-hour urine collection
Day 2	10.00 p.m. finish 24-hour urine collection
Day 3	Morning urine collection Programme day 3

Figure 2A: Urine collection

8.00-9.00	Informed consent procedure Questionnaire 1 Interview
9.00-10.00	BP measurement after 30 minutes of rest Venapuncture (Creatinine, urea, Na, K, PRA.) Breakfast Questionnaire 2
10.00-11.00	Neuromotoric examination Anthropometrical examination Audiometric examination
11.00-12.00	Cold Pressor Test (Pain coping test) IMT measurement by carotic ultrasound
12.00-13.00	Cognition test
13.00-14.00	Evaluation

Figure 2B: Programme day 3
Figure 2: Time line POPS 19 study

DATA OBTAINMENT

In this thesis only parameters on blood pressure and renal function estimation are described in detail.

Blood pressure measurement

SBP and diastolic BP were obtained with an automatic BP device (Dinamap). Three measurements were performed at the nondominant 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 statistical analysis. Mean arterial pressure (MAP) was calculated [(SBP + 2 * DBP) / 3]. Information about medical history and drug use was obtained by an interview.

Renal function

Microalbuminuria Two morning urines were obtained at two different days prior to the visit. Albumin to creatinine ratio (ACR: mg/mmol) was measured in both urine samples. If the mean of two ACR values was higher as 2.2 mg/mmol, the subject was considered as microalbuminuric.[87] When only one morning urine was obtained, that result was used.

Glomerular filtration rate GFR was estimated with the Cockcroft-Gault equation adjusted for body surface area (ml/min/1,73m^2), and with the simplified MDRD equation.[88;89]

Laboratory assays

Sodium, potassium, creatinine, and urea were measured in a fully automated computerized laboratory system with a Hitachi 747 (Hitachi, Tokyo, Japan) chemistry analyzer. Plasma renin activity (PRA) was measured by quantification of the generated Angiotensin I with a radioimmunoassay (Incstar, Stillwater, MN 55082, USA). The sensitivity was 0.05 µg/l per hour and the coefficients of variation ranged from 5.6 to 7.6 % at different levels. Microalbumin was measured with a turbidimetric assay on a Hitachi 911 (Hitachi, Tokyo, Japan).

INFORMED CONSENT AND ETHICS COMMITTEE

Informed consent was obtained after oral and written information had been given. The ethics committees of all participating centers approved the study protocol.

Chapter 3.2

Is BP increased 19 years after intrauterine growth retardation and preterm birth? A prospective follow-up study in the Netherlands

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Pediatrics 2005;116(3):725-731.

ABSTRACT

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

Methods: A prospective follow up study was conducted at age 19 in individuals who were born preterm in the Netherlands in 1983. Systolic, diastolic and mean BP values and plasma renin activity concentration were obtained in 422 young adults born with a gestational age (GA) < 32 weeks. BP values were also measured in 174 individuals who were born with a GA ≥ 32 weeks and a birth weight < 1500 gram.

Results: An increased prevalence of hypertension and probably also of prehypertensive stage was found. IUGR, birth weight, GA and plasma renin activity were not associated with BP. Current weight and BMI were the best predicting factors for systolic BP 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 born, no support to the hypothesis that low birth weight is associated with increased BP 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 ('fetal origins of adult diseases' hypothesis).[90;91] In these studies, an inverse relation has been described between birth weight and hypertension, hyperlipidemia, type II diabetes, and cardiovascular diseases in adult life. Individuals born after intrauterine growth retardation (IUGR) are thought to be at risk for developing high BP (BP) compared with subjects with the same birth weight but no IUGR.[3;92]

Besides low birth weight, three other early factors that are considered to be important risk factors for developing high BP in adult life have been identified in individuals with IUGR. First, accelerated postnatal growth in weight and length are suggested to increase the risk for developing hypertension and type II diabetes in later life, especially in low birth weight individuals.[4;15;16]

Second, it was postulated that altered angiotensin activity was an important factor underlying the 'fetal origins of adult diseases' hypothesis.[58;59] Also, hypoxia increased sympatic nerve activity and catecholamine production and proliferation of juxta-glomerular cells (and thus renin-producing cells) are suggested as factors in the pathogenesis.

Finally, preterm infants are probably at even greater risk of developing adult diseases compared with individuals who were born at term. A large Swedish study showed an inverse association between gestational age (GA), ranging from 35 - 44 weeks, and systolic BP (SBP) in 165,136 Swedish men.[5] This correlation may be stronger in the lower range of gestation (GA 30-38 weeks), as demonstrated by Siewert-Delle et al.[65] Very preterm infants who born with a GA <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 GA: 31 weeks) compared with individuals who were of the same age and born at term.[69]

It is suggested that the underlying mechanism for prematurity influencing BP 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 β-cell development in the pancreas, is probably not or only partly completed after preterm birth.[63] 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 co-morbidity after birth and drug use, are supposed to influence both neonatal and adult health. To our knowledge, no previous prospective studies were able to analyse these potential confounders in the relation to birth weight and BP.

In this article, we describe the results of a large, prospective, follow-up study in which BP was obtained in 19-year-olds who were born in 1983 with a GA <32 weeks. Within this cohort our objective was (1) to determine whether IUGR is associated with increased BP at age 19 after very preterm birth and whether this is amplified as a result of accelerated weight gain and growth postnatally and (2) to determine whether IUGR is associated with alterations in renin concentrations at age 19 after very preterm birth. In addition, the effects of potential maternal, perinatal and postnatal confounders on BP at young adult age were studied, as well as the relation between GA and BP.

METHODS

Study population

Participants were recruited from the POPS cohort (Project On Prematures and Small for gestational age infants). The POPS cohort comprises of 94% of all Dutch neonates (*n*=1338), who were born alive in 1983 with a GA <32 weeks (group 1) and / or a birth weight < 1500 gram (group 2).[86] All individuals who were alive at the age of 19 years (*n*=959) and not lost to follow-up until the age of 14 y (*n*=934) were invited to participate in a prospective follow-up study conducted from April 2002 until May 2003 in ten outpatient clinics in the Netherlands.

Perinatal parameters (*eg*, birth weight, GA, Apgar score, congenital anomalies) and obstetric parameters (*eg*, maternal hypertension, medication during pregnancy, smoking during pregnancy) were known since birth. Follow-up data for growth (height, weight and body mass index) until the age of 10 years were also known in almost all subjects.

Birth weight and birth length were converted to standard deviation scores (BW-sds and BL-sds respectively), using Swedish reference standards.[93] BW-sds was considered as a measure of IUGR. At follow-up visits at the ages 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 weight, length and height values were recorded with standardized scales and were expressed as SDS using Dutch reference standards.[94] BMI was calculated as weight (kg) / length or height $(m)^2$ and converted to SDSs as well for all follow-up ages.

The main statistical analyses included only participants of group 1 (GA < 32 weeks). To study the relation between GA and BP, also participants of group 2 (GA > 32 weeks and birth weight < 1500 gram) were included to increase the range of gestation until 40 weeks. Prevalence rates of hypertension were also calculated in both groups.

Data obtainment

SBP and diastolic BP were obtained with an automatic BP device (Dinamap). Three measurements were performed at the nondominant 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 statistical analysis. Mean arterial pressure (MAP) was calculated [(SBP + 2 * DBP) / 3]. Information about medical history and drug use was obtained by an interview.

Individuals were excluded from the analyses when antihypertensive medication was used, individuals were pregnant, or BP 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 BP (SBP < 120 mmHg and DBP < 80 mmHg), prehypertensive BP (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 or DBP > 100mmHg) according the JNC VII criteria.[95]

A blood sample was obtained after BP measurement, in which plasma renin activity (PRA) was measured by quantification of the generated Angiotensin I with a radioimmunoassay (Incstar, Stillwater, MN 55082, USA). The sensitivity was 0.05 µg/l per hour and the coefficients of variation ranged from 5.6 to 7.6 % at different levels.

Informed Consent and Ethics Committee

Informed consent was obtained after oral and written information had been given. The ethics committees of all participating centers approved the study protocol.

Statistics

Student's unpaired *t* tests were performed to compare BP means. Because birth weight is positively associated with adult weight, and adult weight influences BP (causal pathway)[96], we used a multivariate regression model to analyze the effect of birth weight on BP and the effect of growing more in weight than would be expected from a given birth weight. Therefore, we first used linear regression to calculate the expected adult weight (or weight at 3, 6, 12, 24 months and 5 or 10 years), based on birth weight, and then subtracted the actual adult weight (or weight at the age of 3, 6, 12 months and 2, 5, 10 or 19 years). This 'residual' was entered in the final linear

regression model.[76] Recently, we explained the algebraic concept of this regression model.[97] The coefficient of BW-sds shows the effect of BW-sds on adult BP, and the coefficient of the 'residual adult weight' shows the effect of gaining more weight than expected' on adult BP. Equally, multiple logistic regression analyses were applied to evaluate the effect of BW-sds and residual weight and height adjusted for gender on the prevalence of hypertension. The effect of GA and gender on BP and the prevalence of hypertension was analyzed separately with linear and logistic regression models. Statistical significance was defined at p-value < 0.05.

RESULTS

Subject characteristics

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

Five individuals mentioned that they had had increased BP in the past, but none were treated for hypertension at the time of the study. Eight individuals were excluded from data analysis: 4 because of use of antihypertensive medication for other reasons than hypertension (*eg*, restless legs and nervousness), 2 because of protocol violation, 1 subject because of pregnancy, and 1 because of unreliable BP measurement. Therefore, SBP and DBP data of 588 subjects were analyzed, 264 of whom were male and 324 of whom were female (Table 1). The mean age was 19.29 (range 18.63 -20.18) years. A total of 418 participants were born with a GA < 32 weeks (group 1); 170 were born with a GA ≥ 32 weeks and with a birth weight < 1500 grams (group 2). GA and birth weight of the participants in group 1 were 29.7 ± 1.53 weeks and 1314 ±338 g and in group 2 33.9 ± 1.63 weeks and 1274 ± 177 g, respectively.

Of all individuals who were alive at 19 years, birth weight and GA did not differ between the responders and nonresponders (mean difference for birth weight: 23 g (95%CI:-12;59 g); mean difference for GA: 0.2 week (95%CI:-0.1;0.5)). Baseline characteristics of the nonresponders are shown in table 1. Compared with the individuals 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;134 g) and a longer duration of GA (1.2 weeks; 95% CI of mean difference: 0.9;1.5 weeks).

Table 1: Patient characteristics and Baseline characteristics of non-responders.

	All subjects: *N*=588 < 32 weeks and/or < 1500 gram	Group 1: *N*=418 < 32 weeks	Group 2: *N*=170 ≥ 32 weeks and < 1500 gram	Non responders *N*=363
Men, %	44.9	46.7	41.4	62.8
Birth weight, g	1303 ± 302	1314 ± 338	1274 ± 177	1328 ± 251
GA, wk	30.9 ± 2.5	29.7 ± 1.5	33.9 ± 1.6	31.2 ± 2.6
Age, y	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	

Data expressed by mean ± SD.

BP values

Data of BP values are shown in table 1. The mean (± SD) SBP, DBP and MAP in subjects in group 1 was 123 ± 13 mmHg, 66 ± 8 mmHg, and 85 ± 9 mmHg, respectively. Subjects in group 2 had a mean SBP, DBP, MAP of 122 ± 12 mmHg, 66 ± 8 mmHg, and 85 ± 8 mmHg, respectively. SBP was higher in men (126 ± 12 mmHg) than in women (120 ± 12 mmHg). DBP was lower in men (64 ± 8 mmHg) than in women (68 ± 8 mmHg). 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 infant respiratory distress syndrome status) all were related to BW-sds but not to SBP and DBP values (data not shown). Therefore, no adjustment for these parameters was required.

In a linear regression analysis of participants who were born with a gestational age < 32 weeks, BP was not associated with birth weight, BW-sds, birth length, and BL-sds, all adjusted for gender. Regression coefficients (β values) 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. 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 (β = 2.3 mmHg/ 1 residual weight SDS and 2.4 mmHg / 1 residual BMI SDS, respectively). Early postnatal weight gain (0-2 y) and increase in length were not related to BP at the age of 19 years.

BP was also not related to GA, both when only participants in group 1 were included (β for SBP: -0.251 mmHg / week increase in GA; 95%CI: -1.016;0.514) and when all participants were included (β for SBP: -0.149 mmHg / week increase in GA; 95%CI:-0.542;0.245), or when only subjects with a birth weight < 1500 gram were included (β for SBP: -0.150 mmHg / week increase in GA; 95%CI: -0.553;0.254).

Table 2: Regression coefficients (95%CI) for birth weight (SDS) (BW-sds) and residual weight gain (SDS) at 3, 6, 12, 24 months and 5 and 10 years of age in relation to systolic BP in mmHg (SBP), diastolic BP in mmHg (DBP), and mean arterial pressure in mmHg (MAP) for the participants born with a gestational age < 32 weeks.

	BW-sds	residual weight gain 3 months	residual weight gain 6 months	residual weight gain 12 months	residual weight gain 24 months	residual weight gain 5 years	residual weight gain 10 years	residual weight gain 19 years
SBP	0.500 (-0.667;1.668)	0.556 (-0.375;1.487)	0.696 (-0.395;1.788)	0.667 (-0.439;1.773)	0.383 (-0.790;1.556)	1.736 † (0.708;2.764)	2.212 † (1.102;3.321)	2.324 † (1.433;3.215)
DBP	0.281 (-0.490;1.051)	0.155 (-0.461;0.770)	0.024 (-0.745;0.696)	-0.435 (-1.167;0.296)	-0.519 (-1.294;0.256)	0.013 (-0.667;0.703)	0.395 (-0.373;1.163)	0.165 (-0.441;0.771)
MAP	0.354 (-0.472;1.180)	0.288 (-0.372;0.949)	0.216 (-0.557;0.988)	-0.068 (-0.851;0.715)	-0.219 (-1.051;0.641)	0.588 (-0.149;1.324)	1.000 * (0.194;1.807)	0.885 * (0.240;1.529)

All coefficients are adjusted for gender. Regression coefficients express the increase of BP in mmHg / 1 SDS increase in birth weight (first column) or residual weight gain (remaining columns). Residual weight gain expresses the amount of 'growing more in weight (SDS) than expected given a certain birth weight (SDS)'. * p value < 0.05, † p-value < 0.01

Table 3: Regression coefficients (95%CI) for birth length (SDS) (BL-sds) and residual increase in length or height (SDS) at 3, 6, 12, 24 months and 5 and 10 years of age in relation to systolic BP (SBP), diastolic BP (DBP), and mean arterial pressure (MAP) for participants in group 1.

	BL-sds	residual length 3 months	residual length 6 months	residual length 12 months	residual height 24 months	residual height 5 years	residual height 10 years	residual height 19 years
SBP	0.877 (-0.283;2.037)	0.636 (-0.544;1.817)	0.359 (-0.889;1.606)	0.686 (-0.597;1.970)	0.267 (-1.027;1.580)	0.313 † (0.149;0.478)	1.126 (-0.158;2.409)	0.822 (-0.548;2.192)
DBP	0.641 (-0.121;1.403)	0.258 (-0.509;1.025)	-0.126 (-0.923;0.672)	0.030 (-0.869;0.810)	0.100 (-0.951;0.751)	0.153 * (0.044;0.263)	-0.182 (-1.068;0.703)	-0.400 (-1.017;0.501)
MAP	0.720 (-0.096;1.536)	0.384 (-0.445;1.214)	0.036 (-0.832;0.904)	0.209 (-0.696;1.114)	0.026 (-0.886;0.938)	0.207 * (0.090;2.005)	0.254 (-0.679;1.186)	0.007 (-0.959;0.973)

All coefficients are adjusted for gender. * p value < 0.05, † p-value < 0.01

PRA was inversely related to BP adjusted for gender (β for SBP: -0.022 mmHg / 1 µg/l per hour (95%CI: -0.032;-0.011, p-value 0.001); β for DBP: -0.013 mmHg / 1 µg/l per hour (95%CI: - 0.037;-0.005, p-value 0.011); β for MAP: -0.024 mmHg / 1 µg/l per hour (95%CI: -0.039;-0.009, p-value 0.002). Regression coefficients were not different between participants with low and high BW-sds. PRA was not related with BW-sds or GA (Table 4 for mean values within BW-sds tertiles).

The prevalence of hypertension (SBP > 140 mmHg or DBP > 90 mmHg) was 10.5% and of prehypertensive stage was 45.9% (SBP 120-139 mmHg or DBP 80-89 mmHg) 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;5.3) compared to women (logistic regression). BW-sds and GA both did not affect the risk for hypertension. Increased postnatal weight gain and BMI after 5 years were predictors for the risk for hypertension at the age of 19 years, but current weight (19 years) 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 BP at the age of 19 years, in individuals who were born with a GA < 32 weeks and/or a birth weight < 1500 grams. Our main finding was that we were not able to show a relation between BW-sds, BL-sds, or GA and adult BP. Adjustment for height, a common procedure in pediatric BP interpretation, did not reveal a relation between BW-sds and BP either. Also accelerated postnatal growth or weight gain during the first months in life (postnatal hypothesis) did not influence BP at the age of 19 years. Current weight and BMI were the best predictors for BP at age 19.

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 120 mmHg in women. In comparison, the SBP of subjects of the same age (18-19 years) participating in the Bogalusa heart study was 115 mmHg in white men and 109 mmHg in white women.[98] The Third National Health and Nutrition Examination Survey (NHANES III) study reported a mean SBP in 17-year-olds of 117 mmHg in white boys and 107 mmHg in white girls.[99] So, both in men and women, SBP was higher in our cohort. Other studies, like NHANES and Framingham heart studies, reported mean BP values in larger age categories (29-37 and 18-39 years), making comparison with our results difficult.[100]

The prevalence of hypertension in our study was 10.5%. The overall prevalence of hypertension in individuals between 18 and 39 years of age was 7.2% in the

NHANES III survey.[100] Moreover, as it has been reported that the prevalence of hypertension increases by 1.3% with a 1-year increase of age,[100] the prevalence of hypertension in the general population between 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 twofold risk for progression towards hypertension in later life.[95] Therefore, monitoring of BP 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 to a random 19-year old reference group is not known. Population based reports on BP 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 JNCV criteria.[101] Then, the prevalence of normal BP (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 BP (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 study, respectively) in

Table 4: Mean (SD) of BP values, PRA concentrations and BMI (SDS) within tertiles of birth weight (BW-sds) in participants of group 1.

Subjects group 1: (*N*=418)	Lowest tertile BW-sds (lowest thru –0.3814)	Middle tertile BW-sds (-0.3814 thru 0.3787)	Highest tertile BW-sds (0.3787 thru highest)	ANOVA P-value
SBP, mmHg	122 (13)	123 (12)	124 (13)	0.731
DBP, mmHg	66 (8)	66 (8)	66 (8)	0.914
MAP, mmHg	85 (9)	85 (8)	85 (9)	0.951
PRA, µg/l per hour	2.5 (1.3)	2.2 (1)	2.3 (1.5)	0.331
BMI, SDS	-0.3 (1.3)	-0.2 (1.4)	0 (1.1)	0.074

SBP: Systolic BP, DBP: Diastolic BP, MAP: Mean arterial pressure. PRA: plasma renin activity.

Table 5: Prevalence of hypertension for all participants using the BP (BP) criteria according to the JNC VII.[95]

	All subjects		Group 1: < 32 weeks		Group 2: ≥ 32 weeks and < 1500 gram	
	N	(%)	N	(%)	N	(%)
Normal BP *	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)

* Normal BP: SBP < 120 mmHg and DBP < 80 mmHg.

† Prehypertensive stage: SBP 120-139 mmHg or DBP 80-89 mmHg.

‡ Hypertension stage 1: SBP 140-159 mmHg or DBP 90-99 mmHg.

§ Hypertension stage 2: SBP >160 mmHg or DBP >100 mmHg.

both men and women.[102] Again, the mean age of the participants in our cohort was much lower (19 years in our study versus 29-37 years in the Muscatine study).

Several factors may have influenced our results. First, our protocol of BP measurements to minimize variability deviated from other studies. We measured BP three times in rest, in supine position, and at the nondominant arm. BP 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.[103]

Second, our BP values may have been influenced because of the use of automatic ossilometric device (Dinamap). Two studies comparing manometric and Dinamap BP measurements reported an underestimation of the DBP values between 2.4 and 8.2 mmHg when this automatic device was used.[104;105] 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 1.0-6.7 mmHg when the Dinamap was used.[104;105] When we adjust for the systematic error as reported by Coppieters 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 [104;105] the mean BP in our cohort decreased to 120 ± 12 mmHg for SBP and 67 ± 8 mmHg for DBP in men and 114 ± 12 mmHg for SBP and 70 ± 8 mmHg for DBP in women (data not shown). The prevalence of hypertension within group 1 dropped to 4.3% and of pre-hypertensive stage to 31.8%. Still, mean SBP and DBP were higher compared with the subjects in the NHANES III and Bogalusa heart study.[99;106]

Third, it is recommended that BP be measured at least two times independently before the mean SBP and DBP are calculated a person is defined hypertensive. Our three BP measurements were performed at one day, and are evidently not independent. However, the reference data we used to compare prevalence rates and mean BP values were also based on one initial screening and the mean of at least three measurements.[99;100] Therefore, our data are comparable, showing both high prevalence and high mean BP.

Our study indicates that individuals who were born very preterm have elevated mean BP values and that the prevalence of hypertension is increased at the age of 19 years. This is not related to the extent of IUGR (BW-sds), birth weight (g), or GA. In our cohort the range of birth weight is 560 to 2580 g. Most studies in which the relation between birth weight and adult BP was found included subjects with a birth weight ranging between 2000 and 5000 g.[107-109] This suggests that the relation among birth weight, prematurity and BP may be diminished in the lower birth weight ranges or GAs, and is not a continuously linear but a curved dose-response

relationship. This would explain the increased mean BP values, increased prevalence of hypertension, and the absence of the relation between BW-sds and BP 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 GA of 30 weeks. Future studies may help to confirm our findings.

A few other reasons can account for the absence of the association between IUGR and BP. 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 measure BP 1-2 mmHg accurate). However, changes in BP as a results of IUGR have been shown in other studies at even younger age.[110] Law et al described that the effect of low birth weight on BP may be obscured during adolescence.[12;111] Follow-up of our subjects is therefore recommended.

Second, a selection bias could have been introduced because of a response of 64%. Of all participants who were alive at age 19, no differences in baseline characteristics were present. However, compared to 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 subjects with the suggested highest risk for increased BP were less included in the study, possibly leading to negative results. Even if this bias was introduced and a relation between IUGR and BP was concealed, our results concerning mean BP values and prevalence rates are probably underestimated, and conclusions would not change much.

Finally, it is possible that the relation between birth weight and BP 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. state that most studies that found a relation between birth weight and adult BP included small numbers of subjects.[72] With increasing study size the relation diminished, suggesting an publication bias.[112] Furthermore, most studies failed to account for possible appropriate adjustment for potential confounders, like current weight.[72] As birth weight is positively correlated with current weight and current weight with BP, also in our cohort, current weight cannot be designated as a potential confounder (causal pathway).[70-72] 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.[76] 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 BP.[71;72] In our study we were able to show that maternal hypertension, smoking during pregnancy, neonatal corticosteroids use, presence of infant respiratory distress syndrome, and alterations on cardiotocographic measurement were associated with BW-sds. All of these factors

were not associated with BP at 19 years. We therefore conclude that these parameters are no confounders in our study.

As expected, PRA was negatively correlated to BP. Individuals with high levels of active renin will have lower BP values. However, neither BW-sds nor GA was associated to PRA. These data are not in agreement with the findings of Martyn et al,[59] 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.[58] 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 BP is not present. Contribution to the pathophysiological mechanism of the RAS can therefore not be given.

CONCLUSIONS

In this large ex-preterm cohort, the mean SBP and the prevalence of hypertension were high. 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 BP is a curved dose-response curve needs to be studied, using subjects in all birth weight ranges, gestational ages and sophisticated BP tools.

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Chapter 3.3

Microalbuminuria and lower glomerular filtration rate at young adult age in subjects born very premature and after intrauterine growth retardation

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ABSTRACT

This prospective follow up study in 422 19-year-old subjects born very preterm in the Netherlands was performed to determine whether intrauterine growth retardation (IUGR) predisposes to abnormal glomerular filtration rate (GFR) and microalbuminuria in adolescents. GFR (ml/min/1.73m^2) was estimated, using the Cockcroft-Gault equation, and albumin-creatinine ratio (mg/mmol) was calculated in a cohort of 19-year-old subjects born very preterm (gestational age < 32 weeks) in 1983. Birth weights were adjusted for gestational age and expressed as standard deviation scores (sds) as a measure of IUGR. All subjects had normal renal function. Birth weight (sds) was associated negatively serum creatinine concentration (µmol/l) (β = -1.0 µmol/l, 95%CI: -1.9; -0.2), positively with GFR (ml/min/1.73m^2) (β = 3.0, 95%CI: 1.7; 4.2) and negatively with the logarithm of albumin-creatinine ratio (log mg/mmol) (β= -0.05, 95%CI: -0.09; -0.01) in young adults born very preterm. IUGR is associated with unfavourable renal functions at young adult age in subjects born very premature. These data suggest that intrauterine growth retarded subjects born very premature have an increased risk to develop progressive renal failure in later life.

INTRODUCTION

Fetal kidney development is known to be impaired in subjects liable to intrauterine growth retardation. In both animal and human studies it was shown that neonatal kidney volume and nephron number is reduced when intrauterine growth was impaired.[44;46;113-115] Offspring of Spangue-Dawley rats, who were kept on a low protein diet during pregnancy, had a 28-29% decreased number of glomeruli in proportionally smaller kidneys.[116] Also blood pressure was increased 8 weeks after birth. Mañalich described less glomeruli in the renal cortex per 0.6 mm^2 in low birth weight neonates who died within 2 weeks after birth due to diseases not involving the kidneys.[117] In this study there was a direct relation found between birth weight and number of glomeruli.

It has been suggested that low birth weight and impaired nephron number at birth increases the risk to renal failure and end stage renal disease in later life.[17;18] Brenner et al. suggested that the filtration surface area in kidneys with low nephron number is decreased compared to healthy subjects with normal nephron number. This decrease in filtration surface area would lead to glomerular and systemic hypertension, glomerular damage and sclerosis and therefore to a decrease in renal function ('hyperfiltration theory').[41] One of the first symptoms of developing renal disease is microalbuminuria followed by decrease in glomerular filtration rate.

Recently, data have been published showing a strong relation between birth weight and renal size, nephron number, glomerular volume, albuminuria and systolic blood pressure in Aboriginal communities.[34;45] In communities of aboriginals and Indians diseases like type II diabetes, cardiovascular diseases and renal diseases take epidemic proportions.[42;43] Poverty and poor living conditions leading to low birth weight in the offspring are suggested to be important in the development of these diseases.[118]

It has been shown that preterm born subjects have smaller kidneys than subjects born at term. As nephrogenesis continues until 36 weeks of gestation, subjects born very prematurely are suggested to have less glomeruli than subjects born less preterm at birth. Recently, it was also shown that nephrogenesis ceases after very preterm birth.[63]

The combination of prematurity and intrauterine growth retardation, which often are both present, might increase the risk for progressive renal failure even more. Data on renal function at adult age in subjects born very preterm is very scarce.

We describe the results of a large-scale prospective follow-up study in subjects born with a gestational age < 32 weeks in 1983 in the Netherlands, in which we estimated glomerular filtration rate and measured microalbuminuria at the age of 19 years. The objective was to evaluate the effect of intrauterine growth retarda-

tion in these preterm subjects on renal function at young adult age. In addition, we evaluated the effect of gestational age, in the lowest ranges of gestation, on renal function at young adult age.

METHODS

Study population

In a prospective follow-up study carried out from April 2002 until May 2003 all survivors of the POPS cohort (Project On Prematures and Small for gestational age infants) born with a gestational age < 32 weeks were invited to visit one of ten outpatient clinics in the Netherlands, at the age of 19 years. The POPS cohort was recruited in 1983, where 94% of all Dutch neonates, born alive with a gestational age (GA) < 32 weeks and / or a birth weight (BW) < 1500 gram were included (n=1338).[86] All subjects alive at the age of 19 years (n=959) and not lost to follow up (n=25) were invited to participate in a prospective follow-up study. For this part of the study only subjects born with a gestational age < 32 weeks (n=676) were eligible to participate.

Data obtainment

Two morning urine samples were collected to measure creatinine and microalbumin concentrations to calculate albumin-creatinine ratio (ACR). Female subjects within their menstrual period at time of urine collection were excluded from the analyses of ACR. A blood sample was obtained to measure creatinine, urea, sodium and potassium concentrations.

Glomerular filtration rate (GFR) was calculated both with the Cockcroft-Gault equation, and adjusted for body surface area (ml/min/1,73m^2), and with the simplified MDRD equation.[88;89] ACR (mg/mmol) was calculated in morning urines as a measure of microalbuminuria (<2.2 normal; 2.2-22.6: microalbuminuria; > 22.6 macroalbuminuria or proteinuria).[87] The average ACR in two morning urines was calculated and used in the analyses. When only one morning urine was obtained, that result was used.

Sodium, potassium, creatinine, and urea were measured in a fully automated computerized laboratory system with a Hitachi 747 (Hitachi, Tokyo, Japan) chemistry analyzer. Microalbumin was measured with a turbidimetric assay on a Hitachi 911 (Hitachi, Tokyo, Japan).

Perinatal parameters (like BW, GA, Apgar score) and obstetric parameters (like maternal hypertension, use of medication during pregnancy, smoking during pregnancy) were derived from the original POPS database (TNO Quality of Life, Leiden,

The Netherlands). These parameters were obtained in 1983 directly after birth. Gestational age (best obstetric estimate) was based on last menstrual period, pregnancy testing, and ultrasound (if necessary). This was available in 1335 cases. The reliability of this estimate was stated to be excellent in 78% of cases; in only 7% there was a discrepancy with the pediatric assessment of 2 weeks or more (pediatric maturity score = Dubowitz score), which is well within the variability of the scoring systems.[86].

Birth weight adjusted for gestational age and adult weight were converted to standard deviation scores (BW-sds), using Swedish and Dutch reference standards.[93;94] Subjects with a BW-sds < 0 were defined as small for gestational age (SGA) and subjects born with a BW-sds ≥ 0 were defined as appropriate for gestational age (AGA). BW-sds was considered as a measure of intrauterine growth retardation.

Informed Consent and Ethics Committee

Informed consent was obtained after oral and written information had been given. The ethics committees of all participating centers approved the study protocol.

Statistics

Standard methods were used for calculation of the mean, the standard deviation (SD), standard error of the mean (SEM), and the 95% confidence intervals (95%CI). To study the effect of BW-sds on renal function, independent of adult weight (sds) we used a multivariate regression model. In fact, we were interested in the effect of 'growing more than would be expected from a given birth weight'. Therefore, we first calculated the expected adult weight (sds), based on BW-sds, and then subtracted the actual adult weight (sds). This 'residual' was entered in the regression model [76] leading to an effect of adult weight (sds) independent of BW-sds. The algebraic concept of this regression model is recently explained[97]. The coefficient of BW-sds shows the effect of BW-sds on the outcome, and the coefficient of the 'residual adult weight' shows the effect of gaining more weight than expected' on the outcome. In all regression models we adjusted for gender. Statistical significance was defined when the p-value was < 0.05.

RESULTS

Of 676 eligible subjects 422 participated (46.7% males) in this study (response rate 62.4%). Subject characteristics are shown in table 1. The mean (SD) age was 19.3 (0.2) years. The mean (SD) birth weight was 1317 (338) gram, gestational age 29.7 (1.5) weeks and the BW-sds –0.11 (1.02). Sodium, potassium and urea concentrations were in normal range in all subjects and equal in the SGA and AGA group.

Table 1: Patient characteristics.

	All subjects: N = 422	SGA (BW-sds < 0) N = 215	AGA (BW-sds ≥ 0) N = 207	P value (t-test)
Age (yrs)	19.3 (0.2)	19.3 (0.2)	19.3 (0.2)	0.323
Males (number (%))	197 (46.7%)	91 (42.3%)	106 (51.2%)	0.068 (χ^2)
BW (gram)	1317 (338)	1144 (259)	1496 (317)	< 0.001
GA (weeks)	29.7 (1.5)	30.0 (1.4)	29.4 (1.6)	< 0.001
BW-sds	-0.11 (1.0)	-0.87 (0.78)	0.68 (0.49)	< 0.001
BMI*	21.74 (3.4)	22.01 (3.4)	21.94 (3.3)	0.113
Serum creatinine (µmol/l) *	81.8 (10.2)	81.9 (9.8)	81.7 (10.6)	0.862
CG GFR (ml/min/1.73m^2) *	107.0 (15.8)	105.1 (16.0)	108.9 (15.4)	0.018
MDRD GFR (ml/min/1.73m^2) *	98.0 (14.7)	96.6 (13.6)	99.4 (15.8)	0.064
Serum urea (mmol/l) *	4.6 (1.1)	4.6 (1.1)	4.6 (1.2)	0.739
Serum sodium (mmol/l) *	143 (3)	143 (3)	143 (3)	0.681
Serum potassium (mmol/l) *	4.5 (0.4)	4.5 (0.4)	4.5 (0.5)	0.919
log ACR (log(mg/mmol)) *	-0.69 (0.40)	-0.74(0.36)	-0.64 (0.44)	0.015
Geometric ACR (mg/mmol)	0.20 (2.52)	0.18 (2.27)	0.23 (2.77)	

Data expressed by mean ± standard deviation, except when notified differently.
SGA: Small for gestational age; AGA: Appropriate for gestational age; BW: birth weight; GA: gestational age; BW-sds: birth weight adjusted for gestational age expressed by standard deviation scores; CG GFR: Cockroft-Gault glomerular filtration rate; MDRD GFR: simplified MDRD glomerular filtration rate; ACR: albumin creatinine ratio (log ACR values and the geometric values of these log transformations are shown).
P-values based on Student's t-test, comparing AGA and SGA subjects. In the distribution of males and females χ^2-test was used.
* Number of subjects ranges between 368 and 414.

Regression coefficients (adjusted for gender) of birth weight (both SDS and grams) and gestational age on renal function parameters are summarized in table 2 and 3. The effect of adult weight (SDS) independent on BW-sds is also mentioned in table 2.

Creatinine concentration and glomerular filtration rate

The mean (SD) serum creatinine concentration (n=396) was 81 (10) µmol/l. All values were within normal range (51-115 µmol/l). Serum creatinine concentration was inversely related to BW-sds (β = -1.0; 95%CI -1.9; -0.2); so, serum creatinine concentration was the highest in subjects with lowest birth weight (for gestational age). This relation was equal in males and females. However, creatinine concentration was 10.64 µmol/l higher in males compared to females (95%CI for mean difference: 8.9;12.4, p-value < 0.001). Figure 1 shows the relation between birth weight (SDS) and serum creatinine concentration in males (Figure 1A) and females (Figure 1B).

Cockcroft-Gault GFR was available for 388 subjects. The mean (SD) GFR was 107.0 (15.8) ml/min/1.73m^2 ranging between 76.7 and 210.7 ml/min/1.73m^2. The mean GFR was 6.4 ml/min/1.73m^2 higher in males than in females (95%CI 3.3-9.5). When the MDRD equation was used, the mean GFR (SD) was 98.0 (14.7) ml/min/1.73m^2,

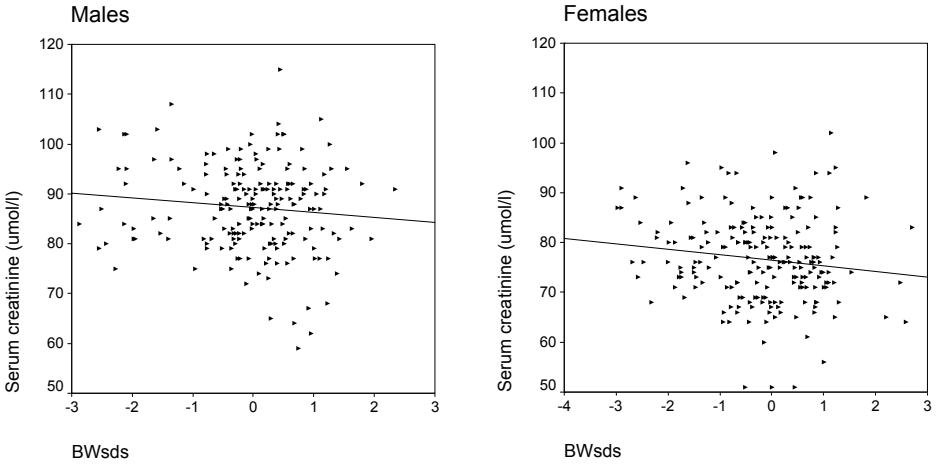

Figure 1A and 1B: Scatterplot between birth weight (sds) (BW-sds) and serum creatinine concentration in males (1A) and females (1B) at the age of 19 years.

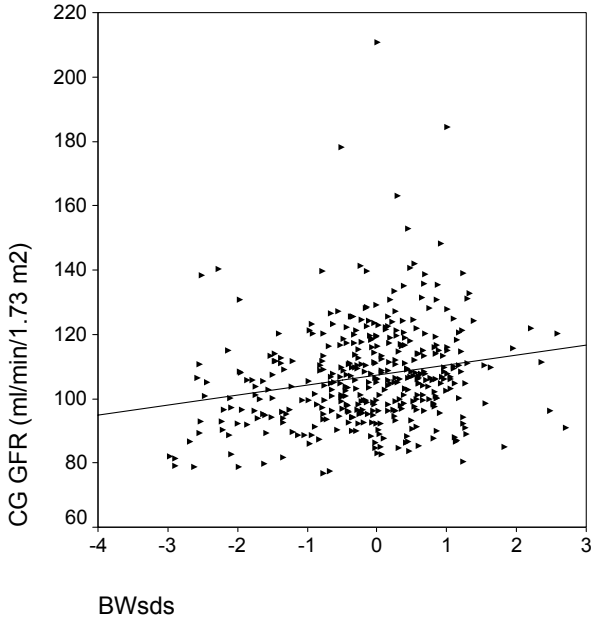

Figure 2: Scatterplot between birth weight (sds) (BW-sds) and Cockroft-Gault glomerular filtration rate (CG GFR) at the age of 19 years.

Table 2: Regression coefficients of birth weight SD (BW-sds) with 95% confidence intervals (95%CI) and p values adjusted for residual adult weight and gender.

	Coefficient of BW-sds	95% CI	P-value	Coefficient of residual adult weight (sds)	95% CI	P-value	Mean increase in males	95% CI	P-value
Serum creatinine (µmol/l)	-1.046	-1.886; -0.206	0.015	0.146	-0.510; 0.801	0.663	11.067	9.380; 12.753	< 0.001
CG GFR (ml/min/1.73m^2)	2.954	1.665; 4.243	<0.001	6.240	5.236; 7.245	< 0.001	6.24	5.236; 7.245	< 0.001
MDRD GFR (ml/min/ 1.73m^2)	1.230	0.003; 2.458	0.050	-0.127	-1.085; 0.831	0.794	13.587	11.122; 16.052	< 0.001
log ACR †	-0.051	-0.092;-0.010	0.016	-0.005	-0.037; 0.027	0.758	- 0.002	-0.085; 0.080	0.958,

CG GFR: Glomerular Filtration Rate (ml/min/1.73m^2) calculated with Cockroft-Gault equation.
MDRD GFR: Glomerular Filtration Rate (ml/min/1.73m^2) calculated with MDRD equation.
Log ACR: Logarithm of Albumin Creatinine Ratio (mg/mmol).
Interpretation of the equation for ACR: ACR (mg/mmol) = $10^{(-0.051 * \text{BW sds})}$ / $10^{(-0.005 * \text{residual adult weight})}$ / $10^{-0.002}$ if male.
† Without adjustment for residual weight and gender, the coefficient and 95%CI for BW-sds to log ACR is nearly equal (-0.050, 95%CI: -0.091 and –0.010).

Table 3: Regression coefficients of birth weight and gestational age on outcome with 95% confidence intervals (95%CI) and p values adjusted for gender .

	BW (per 100 grams)	95% CI	P-value	GA (weeks)	95% CI	P-value
Serum creatinine (µmol/l)	-0.385	-0.642; -0.127	0.004	-0.171	-0.741; 0.399	0.556
	in males: + 11.083	9.359; 12.806	< 0.001	in males: + 10.636	8.921; 12.352	< 0.001
CG GFR (ml/min/1.73m^2)	1.129	0.672; 1.586	< 0.001	0.854	-0.167; 1.875	0.101
	in males: + 5.153	2.102; 8.204	0.001	in males: + 6.428	3.341; 9.515	< 0.001
MDRD GFR (ml/min/1.73m^2)	0.412	0.027; 0.797	0.036	0.023	-0.826; 0.872	0.957
	in males: + 14.314	13.839; 11.263	< 0.001	in males: + 14.314	11.762; 16.866	< 0.001
log ACR	-0.019	-0.031; -0.006	0.003	-0.015	-0.042; 0.012	0.288
	in males: + 0.016	-0.073; 0.094	0.802	in males: + 0.012	-0.095; 0.070	0.773

BW: coefficient of actual birth weight (per 100 gram) on outcome.
GA: coefficient of gestational age (per week) on outcome.
Interpretation of the equation for ACR and BW: ACR (mg/mmol) = 10(-0.019*BW per 100 grams) * 100.016 if male.

ranging between 64.2 and 162.5 ml/min/1.73m^2. Also the MDRD GFR was 14.3 ml/min/1.73m2 higher in males than females.

Both Cockroft-Gault GFR and MDRD GFR were positively related to BW-sds (Figure 2), meaning that subjects with low birth weight (for gestational age) have lower GFR values than subjects with high birth weight (for gestational age). The coefficient for the CG equation was 3.0 ml/min/1.73m^2 (95%CI 1.7; 4.2) and for the MDRD equation 1.2 ml/min/1.73m^2; 95%CI 0.0; 2.5). Also adult weight (sds) was, independent of birth weight, highly associated to CG GFR (β = 6.2; 95%CI 5.2;7.2) but not to MDRD GFR (β = -0.1; 95%CI –1.1;0.8).

Birth weight was also related to serum creatinine concentration and GFR. An increase of 100 grams in birth weight corresponded with a decrease of serum creatinine concentration of 0.39 µmol/l (95%CI: -0.642;-0.127) and an increase in GFR of 1.129 ml/min/1.73m^2 (95%CI: 0.672;1.586).

Creatinine concentration and GFR were not related to gestational age (β = -0.2; 95%CI –0.7; 0.4 for creatinine concentration and β = 0.9; 95%CI –0.2; 1.9 for GFR). Data are shown in table 3.

Microalbuminuria

One or two morning urines were available in 404 subjects. Thirty-one females were excluded from data analyses because the urine was obtained within their menstruation period. In 5 subjects data on laboratory results were missing, leaving 368 subjects for data analyses. In 77 subjects only 1 morning urine was available for analyses. The mean ACR did not differ from the average ACR in subjects with 2 available morning urines (mean difference 0.14 mg/mmol, 95%CI: -0.29;0.56).

The prevalence of microalbuminuria (ACR > 2.2 mg/mmol) in the total group was 2.7% (Table 4). The prevalence in SGA subjects (3.8%) was 2.4 times higher (95%CI 0.6-9.3) than in the AGA subjects (1.6%). The mean (SD) ACR in SGA subjects was 0.51 (1.43) mg/mmol and in the AGA subjects 0.29 (0.54) mg/mmol (mean difference 0.21 mg/mmol (95%CI: –0.01;0.44)). This trend was confirmed in a regression model, showing that subjects with lower birth weights (sds) had higher ACR values compared to subjects with higher birth weights (sds) (β = -0.11 95%CI –0.22; 0.01). As the distribution of ACR was left-sided skewed (mean (SD): 0.40 (1.09), range: 0.04;15.14, and skewness 9.3), we examined whether the relation remained present after logarithmic transformation (log-ACR mean (SD): -0.7 (0.4), range: -1.44; 1.18, and skewness 1.3). Birth weight (sds) was still significantly related to ACR (ACR = $10^{-0.05 * BW\ sds}$, 95%CI for β: -0.09; -0.01) as shown in figure 3 (p=value 0.02). ACR also decreased with increase of actual birth weight (per 100 gram) (ACR = $10^{-0.02 * BW\ per\ 100\ gram}$, 95%CI for β: -0.03; -0.01). Gestational age was not related to microalbuminuria in our study (Table 3).

Table 4: Distribution of microalbuminuria in morning urines

	All subjects		SGA (BW-sds < 0)		AGA (BW-sds ≥ 0)	
	Number	%	Number	%	Number	%
No microalbuminuria (ACR < 2.2)	358	97.3%	178	96.2%	180	98.4%
Microalbuminuria (ACR > 2.2 and ACR < 22.6)	10	2.7%	7	3.8%	3	1.6%
Total	368	100%	185	100%	183	100%

SGA: Small for gestational age; AGA: Appropriate for gestational age; BW-sds: birth weight adjusted for gestational age expressed by standard deviation scores; ACR: albumin creatinine ratio

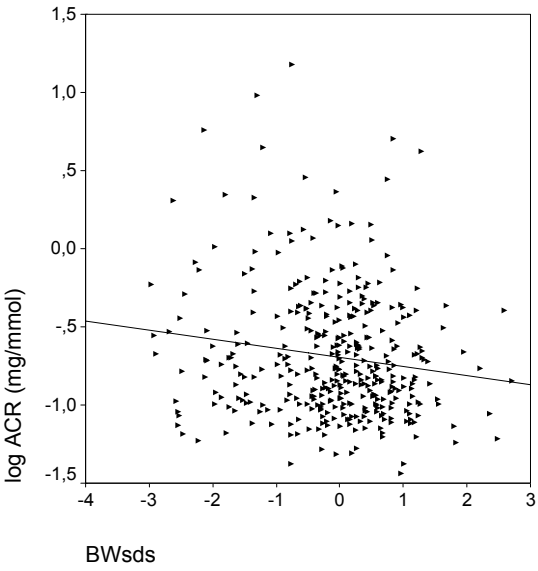

Figure 3: Scatterplot between birth weight (sds) (BW-sds) and the logarithm of Albumin creatinine ratio (ACR) at the age of 19 years.

DISCUSSION

We have found an association between the extent of intrauterine growth retardation and renal functions of young adults born very prematurely (GA < 32 weeks). On average, our subjects born with low birth weights (sds) had lower GFR, higher serum creatinine concentration and higher microalbumin excretion at the age of 19 years. These associations were independent on adult weight (sds).

Controversy exists which equations for GFR estimates real GFR best. The Cockroft-Gault (CG) equation is suggested to be more accurate in healthy subjects with normal GFR values, but is dependent on adult weight.[119] The (simplified) MDRD formula is not dependent on adult weight, but is based on patients with renal dysfunction and therefore suggested as less reliable in healthy individuals. To estimate GFR the CG equation is the most common used in clinical settings in adults. Adult weight was a very strong predictor for CG GFR, acting as a factor in the causal pathway when multivariate regression analysis is used. Using the multivariate regression analysis with residuals, as explained in the statistical section, the influence of this causal pathway factor was prevented. The relation between birth weight (sds) and GFR we found was therefore not influenced by adult weight (sds). Moreover, both CG and MDRD GFR estimations show a positive significant relation between BW-sds and GFR, with a 1.2 to 3.0 ml/min/1.73m^2 increase in GFR per 1 BW-sds increase.

In this study also actual birth weight (grams) was related to renal outcome at 19 years. However, no convincing relation between gestational age (up to 32 weeks of age) and renal function was found. Therefore, it is most likely that not actual birth weight (grams) is the important predicting factor for renal outcome, but indeed the extent of intrauterine growth retardation (birth weight SDS). If not, the relation between GA and renal function would have been much stronger.

A potential selection bias may have been introduced as a result of a response of 62.4%. Non-responders were hard to trace or not willing to participate, mainly due to a lack of time, lack of interest or fear of medical examination. Unfortunately, specific information on renal function, renal diseases and renal deaths in non-responders were not available. However, BW, GA and BW-sds were not significantly different between responders and non-responders. So, therefore the effect of a potential selection bias on our results is probably limited.

Our findings suggest the possibility that the normal process of aging, with a decline of GFR after the age of 20 years [120], may be enhanced after IUGR and preterm birth. We cannot predict if and how much the relation between birth weight (SDS) and renal function will strengthen with age. To study the effect on the incidence of renal function decline and renal disease in later life follow-up of our cohort is recommended.

Our results are in agreement with several animal studies, showing that renal function is decreased after IUGR.[46;121] Also, several human studies have linked IUGR with renal function.[17;18;45] In the Saskatchewan population in Canada women with ESRD were 3 times more likely to have had low birth weight compared to subjects without ESRD.[17] In this study increased in maternal age, known as a risk factor for prematurity and low birth weight was also related to ESRD of the offspring at adult age. Lackland described a U-shaped quadratic association between birth weight

and early-onset of chronic renal failure in subjects living in the southeast of the United States (US).[18] In this study, neither differences in gender nor race was seen. However, as low birth weight is suggested to be more common in black people and in the Southeastern part of the US, this may explain the increased prevalence of ESRD in that region. Another recent study in 668 aboriginal subjects between 4 and 72 years showed that subjects with the lowest birth weights had smallest kidneys (measured by ultrasound), and the subjects with the smallest kidney size had the highest blood pressure and the highest rates of albuminuria (ACR ≥ 34 mg/mmol).[34] Also, nephron number was inversely related to glomerular volume, suggesting that glomerulomegaly is a marker of an increased risk of groups of patients or populations with progressive renal disease.[44] These studies all show that in these populations at risk for ESRD birth weight is lower, supporting the 'hyperfiltration theory' as a possible mechanism saying that IUGR is a risk factor for the development of progressive renal disease.[41]

In our study the overall prevalence of microalbuminuria was 2.7%. This prevalence was two times higher in SGA subjects than in AGA subjects. In a linear regression analysis we confirmed a relation between birth weight and microalbuminuria. SGA subjects have an increased risk for higher microalbumin concentrations, and therefore may be at risk to develop real microalbuminuria later in life.

The relations between birth weight (sds) and renal function in our study are weak, and do not have clinical implications for the subjects at this age. The knowledge of decrease in glomerular filtration rate after the age of 20 years and the increase in the prevalence of microalbuminuria, and therefore the risk for developing progressive renal disease, shows the importance of these small differences at this young age.[120;122] Follow-up of our cohort is recommended to trace subjects with early decrease in renal function and to study whether the relation between birth weight (sds) and renal function will straighten with increase of age.

In conclusion, our data support the hypothesis that in subjects born prematurely IUGR affects renal development in an unfavourable way, possibly leading to progressive renal failure in later life.

ACKNOWLEDGEMENTS

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Chapter 4

POPS Nephrology study: Study design, blood pressure, renal function, and renal ultrasound

Chapter 4.1

Study design POPS Nephrology study

STUDY POPULATION

Three groups of young adults were recruited to participate in our study. The individuals in the first group (*n*=23) were born very premature (< 32 weeks) and small for GA (SGA). The second group (*n*=29) consisted of individuals who were born very premature, but appropriate for GA (AGA). Individuals in the third group (*n*=30) were all born full term and appropriate for GA (Controls).

The SGA and AGA group were recruited from the database of the Project of Preterms and Small for GA infants (POPS cohort) as described in chapter 3.1 of this thesis. The cohort contains 94% of all individuals born alive in 1983 in the Netherlands with a GA < 32 weeks and/or a birth weight < 1500 grams (*n*=1338). In 2002-2003 62.4% of the individuals who were alive (*n*=934) at the age of 19 years participated in the POPS 19 study (*n*=596). In our new study *only* individuals who participated in the POPS 19 study and who were born with a GA <32 weeks were eligible for inclusion in the POPS Nephrology study (*n*=422).

Perinatal parameters (such as BW, GA, Apgar score) and obstetric parameters (such as maternal hypertension, use of medication during pregnancy, smoking during pregnancy) of these prematurely born individuals were derived from the original POPS database (TNO Quality of Life, Leiden, The Netherlands). These parameters were obtained in 1983 directly after birth in all preterm born participants. These data were not available in controls.

GA (best obstetric estimate) was based on last menstrual period, pregnancy testing, and ultrasound (if necessary). The reliability of this estimate was stated to be excellent in 78% of cases; in only 7% there was a discrepancy with the pediatric assessment of 2 weeks or more (pediatric maturity score = Dubowitz score), which is well within the variability of the scoring systems.[86] Birth weight was converted to standard deviation scores adjusted for GA (BW-sds) using Swedish reference standards[93] and considered as a measure of IUGR. To convert adult weight in standard deviation scores Dutch reference standards were used.[94]

Preterm born SGA individuals had a BW-sds < -2 SDS and preterm born AGA individuals had a BW-sds < 2 but > 0 SDS. As the eligible AGA group of individuals was large (*n*=205), the individuals living close to the hospital were recruited first.

The control group consisted of students born between January 1st 1982 and December 31st 1984. They were recruited in the hospital region by advertisement. Data on birth weight and GA in the control group were collected by birth records, or when unavailable by mother's recall. Individuals with a GA < 37 weeks or a birth weight < 2500 gram were excluded from the study.

Exclusion criteria for all participants were 1) the use of antihypertensive medication, 2) contraindication for the use of plastic cannula, or study medication (Inulin, PAH or dopamin), females within their menstrual period of pregnancy, and vegetarians.

DATA OBTAINMENT

Participants were asked to visit the ErasmusMC-Sophia Children's Hospital during 1 day. Prior to this visit the participant was asked to collect 3 morning urines. Ambulant blood pressure monitoring (ABPM) was obtained after visiting the hospital during 24 hours. A time line for study protocol is given in Figure 1.

Day 1	Morning urine collection
Day 2	Morning urine collection
Day 3	Morning urine collection Programme day 3
	3.45 p.m. Start 24 hour ambulant blood pressure monitoring
Day 4	3.45 p.m. Finish 24 hour ambulant blood pressure monitoring

Figure 1A: Urine collection en blood pressure monitoring

8.00-9.00	Informed consent 3 baseline blood pressure measurements Saline infusion 10 ml/kg/h + blood withdrawal (Creatinine, urea, Na, K)
9.00-10.00	Renal ultrasound Inulin and PAH bolus injection (45 mg/kg Inulin and 8 mg/kg PAH) Start continuous infusion Inulin and PAH (42 mg/min Inulin and 20 mg/min PAH)
10.00-11.00	Start baseline renal function test (t=0): voiding or blood withdraw every 15 minutes and 2 blood pressure measurements
11.00-12.00	Renal function test
12.00-13.00	Lunch
13.00-14.00	Start dopamine infusion (2µg/kg/min) Start stimulated renal function test (t=150): voiding or blood withdraw every 15 minutes and 2 blood pressure measurements
14.00-15.00	Renal function test
15.00-16.00	Renal function test Start 24 hour ambulant blood pressure monitoring

Figure 1B: Programme day 3
Figure 1: Time line POPS Nephrology study

Baseline blood pressure

Three baseline BP measurements (minimal interval 1 minute) in rest, at the non-dominant arm, in sitting position were obtained using a calibrated automatic blood pressure device (Hewlett Packard). The mean of the three baseline BP measurements were calculated and compared with the results of the ABPM (see below).

Blood samples

Blood samples were withdrawn to measure creatinine, urea, Na and K directly and to measure renin concentration. Timed blood samples to measure Inulin, PAH and Na concentrations were withdrawn during the renal function test.

Renal ultrasound

Renal ultrasound was performed by one of the three pediatric radiologists according a standardized protocol. Subjects were measured in prone position with a Philips Zs2U or ZSJ5 scanner with a C5-2 curved array transducer (Philips BV Nederland, The Netherlands).

Measures of maximal bipolar kidney length, width and thickness were obtained for both right and left kidney. Renal width and thickness were measured at the level of the kidney hilum. Renal volume (ml) was calculated using the formula (π / 6) * length (cm) * width (cm) * thickness (cm). Cortical thickness was defined as the distance between the external surface of the kidney and top of the medulla. As absolute renal size is known to be associated with body size, we calculated the relative kidney length and volume (RKL and RKV respectively).[123] RKL was calculated dividing renal length by body height. Relative kidney volume was calculated dividing renal volume by body surface area (√ (body height (m) * weight (kg)) / 3600). Renal and ureterovesical anomalies were described.

Renal function

Microalbuminuria Three morning urines were obtained at three consecutive days prior to the renal function test to measure albumin creatinine ratio (ACR: mg/mmol). If the mean of three ACR values was higher as 2.2 mg/mmol, the subject was considered as microalbuminuric.[87]

Baseline renal function Subjects were instructed to have a light, fructose free breakfast. Baseline GFR and ERPF were estimated by the mean of three 30 minutes clearance periods with respectively Inulin (Inutest 25%, Fresenius, Austria) and ρ-aminohippuric acid (PAH: sodium aminohippurate 20%, MSD). A single bolus injection of 45 mg/kg Inulin and 8 mg/kg PAH preceded the continuous infusion of 42 mg/min Inulin and 20 mg/min PAH. Subjects were instructed to void 45 minutes (t=0) after the bolus injection and at t=30, t=60 and t=90 minutes. Blood samples

were taken, from the opposite arm where medication was infused, in the middle of each clearance period at t=15, t=45 and t=75 minutes.

Stimulated renal function After baseline renal function test, the subjects were given a protein rich lunch.124;125 Forty minutes after lunch, a low dose dopamin infusion was started at a rate of 2µg/kg/min.124 After five minutes three new clearance periods were started: Voiding at t=150, t=180, t=210, and t=240 and blood samples at t=165, t=195, and t=235.

Calculation of GFR, ERPF and Fractional sodium excretion GFR and ERPF adjusted for body surface area were calculated using the equation: $U_{Inul/PAH}$ * Volume (ml) * 1.73 / $P_{Inul/PAH}$ * duration (min) * BSA, in which $U_{Inul/PAH}$ and $P_{Inul/PAH}$ reflects the concentration (mg%) of Inulin or PAH in the urine and plasma respectively, and BSA the body surface area (√ height (m) * weight (kg) / 3600). The filtration fraction (FF) was calculated by dividing GFR by ERPF (*100%).

To monitor systemic effects of dopamin infusion, we obtained systolic and diastolic blood pressure (SBP, DBP) during the test. Also fractional urinary excretion of sodium (FeNa) was evaluated before and after renal stimulation, by dividing the sodium clearance by the Inulin clearance.

ABPM

After the renal function tests were finished, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured with a Spacelabs™ 90207 ambulant blood pressure monitor (ABPM) at the nondominant upperarm during 24 hours. Cuff size was adjusted for upperarm length as described previously.[103] Participants were instructed to keep their arm steady during measurement. Reading frequency was programmed for every 20 minutes from 7 AM to 11 PM (daytime) and for every hour between 11 PM and 7 AM (night time). Mean daytime, nighttime and overall BP values and absolute and relative differences in day and nighttime BP were calculated within the three groups. Mean arterial pressure was calculated using (SBP + 2* DBP)/3. Individuals were categorised BP stages according the JNC VII criteria.[95]

Laboratory assays

Analyzes were determined on a Hitachi 917 (Roche Diagnostics). Electrolytes were measured by using Ion Selective Electrodes. Enzymatic assays were used for the determination of creatinine (CREA Plus®) and urea in urine and serum. For the determination of microalbumin in urine an immunoturbidimetric assay was used. Inulin and PAH concentration was measured in all renal test blood and urine samples using the automatic Anthrone and Bratton-Marshall technique.[126;127] Renin (ng Al/mg per hour) was quantitated by its capacity to generate angiotensin I from excess substrate angiotensinogen, measured by an in-house radioimmunoassay, as described previously.[128]

INFORMED CONSENT AND ETHICS COMMITTEE

Informed consent was obtained after oral and written information had been given. The ethics committee of the Erasmus MC – Sophia Children's Hospital, University Medical Center Rotterdam approved the study protocol.

Chapter 4.2

Very preterm birth is risk factor for increased systolic blood pressure and prehypertension at young adult age

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MG Keijzer-Veen, A Dülger, FW Dekker, J Nauta, AJ van der Heijden

Submitted for publication

ABSTRACT

Objective: This study was conducted to investigate the effect of prematurity and IUGR on young adult blood pressure.
Design: Prospective follow-up of very preterm born individuals at the age of 20 years compared with controls.
Setting: Assessing risk factors for primary care.
Participants: We included 29 appropriate and 21 small for GA (AGA, SGA) very premature individuals (GA < 32 weeks), and 30 appropriate birth weight and full term born controls (37-42 weeks) at 20 years of age.
Interventions: Ambulant blood pressure was monitored during 24 hours at the non-dominant arm with a Spacelabs™ 90207 device.
Main outcome measures: Daytime blood pressure values (7 AM to 11 PM) measured with a 20-minute interval and night time blood pressure values (11 PM to 7 AM) measured with a 60-minutes interval.
Results: The mean (SD) daytime SBP in SGA and AGA individuals was 122.7 (8.7) and 123.1 (8.5) mmHg respectively, and 3.6 mmHg (95%CI:-0.9;8.0) versus 4.2 mmHg (95%CI:0.4;8.0) higher than in controls (119.6 (7.6)). Daytime DBP and night time SBP and DBP were equal. Elevated blood pressure (>140/90) was 1.6 times (95%CI:1.0;2.4) more common in AGA preterms compared with controls.
Conclusions: We conclude that very preterm born individuals have higher daytime adult systolic blood pressure and higher risk of hypertension at young adult age.

INTRODUCTION

In the last decades it has frequently been reported that intrauterine growth retardation (IUGR) affects the normal development of organs, such as the kidney, the pancreas and the vascular system, leading to an increased risk for hypertension and / or cardiovascular diseases.[3;12;90;92]

Impaired fetal kidney development is considered as one important pathway in the development of hypertension after IUGR birth.[116;129;130] IUGR is associated with a decreased number of nephrons, leading to a decreased filtration surface area, which may lead to hyperfiltration, glomerular hypertension, glomerular damage, and changes in the renin angiotensin system (RAS) with an increase in systemic BP.[41;58;59]

In addition to IUGR, premature birth may also contribute to the development of adult diseases.[5;65-67;131] In very premature born neonates many organ systems, such as lungs, pancreas, and kidneys are not fully developed yet. After premature birth the postnatal organ development may not be equal compared with the prenatal organ development in individuals born at term, but data are limited.[63] Recently, we have demonstrated unfavorable renal function, but equal blood pressure at the age of 19 years in very preterm born individuals born with IUGR compared with very preterm born individuals with appropriate birth weight.[132;133] It was suggested that the prevalence of hypertension was increased, but controls were lacking.

In this article we describe the results of a prospective follow-up study at the age of 20 years, in individuals who were born very premature and in controls born after full gestation. We obtained renin concentrations and BP. BP was measured both by an initial screening and by a 24-hour ambulant blood pressure monitor. The first objective of the study was to determine whether IUGR was associated with increased BP within young adults born very preterm. The second objective was to determine the effect of gestational age (GA) on BP at young adult life. Third, we evaluated the renin concentration in these individuals in relation to BP.

METHODS

Study population

Three groups of young adults were recruited to participate in our study. The individuals in the first group were born very premature (< 32 weeks) and small for GA (SGA). The second group consisted of individuals who were born very premature, but appropriate for GA (AGA). Individuals in the third group were all born full term and appropriate for GA (Controls).

The SGA and AGA group were recruited from the database of the Project of Preterms and Small for GA infants (POPS cohort) [86]. This cohort contained 94% of all individuals born alive in 1983 in the Netherlands with a GA < 32 weeks and/or a birth weight < 1500 grams (*n*=1338). In 2002-2003 62.4% of the individuals who were alive (*n*=934) at the age of 19 years participated in the POPS 19 study (*n*=596).[133] In our new study *only* individuals who participated in the POPS 19 study and who were born with a GA <32 weeks were eligible for inclusion (*n*=422).

Perinatal parameters (such as BW, GA, Apgar score) and obstetric parameters (such as maternal hypertension, use of medication during pregnancy, smoking during pregnancy) were derived from the original POPS database (TNO Quality of Life, Leiden, The Netherlands). These parameters were obtained in 1983 directly after birth in all preterm born participants. These data were not available in controls.

GA (best obstetric estimate) was based on last menstrual period, pregnancy testing, and ultrasound (if necessary). The reliability of this estimate was stated to be excellent in 78% of cases; in only 7% there was a discrepancy with the pediatric assessment of 2 weeks or more (pediatric maturity score = Dubowitz score), which is well within the variability of the scoring systems [86]. Birth weight was converted to standard deviation scores adjusted for GA (BW-sds) using Swedish reference standards [93] and considered as a measure of IUGR. To convert adult weight in standard deviation scores Dutch reference standards were used [94].

For the SGA group all individuals of the POPS 19 study group with the lowest BW-sds (<-2) were asked to participate in our study (n=26). For the AGA group individuals with a BW-sds between 0 and 2 were asked to participate, starting with the highest BW-sds. As the number of eligible individuals in the AGA group was very large (n=205), the individuals living closer to the hospital were recruited first. The control group, containing students born between January 1st 1982 and December 31st 1984, was recruited in the hospital region by advertisement. Data on birth weight and gestational age in the control group was collected by birth records or, when unavailable, by mother's recall.

Data collection

Three baseline BP measurements (minimal interval 1 minute) after at least 15 minutes of rest, at the nondominant arm, in sitting position were obtained using a calibrated automatic blood pressure device (Hewlett Packard). Thereafter, systolic blood pressure (SBP) and diastolic blood pressure (DBP) was measured with a Spacelabs™ 90207 ambulant blood pressure monitor (ABPM) at the nondominant upperarm during 24 hours. Cuff size was adjusted for upperarm length as described previously.[103] Participants were instructed to keep their arm steady during measurement. Reading frequency was programmed for every 20 minutes from 7 AM to

11 PM (daytime) and for every hour between 11 PM and 7 AM (night time). Mean daytime, night time and overall BP values and absolute and relative differences in day and night time BP were calculated within the three groups. Mean arterial pressure was calculated using (SBP + 2* DBP)/3. Individuals were categorised BP stages according the JNC VII criteria.[95]

A blood sample was withdrawn in rest, after baseline BP measurement. Renin (ng Al/mg per hour) was quantitated by its capacity to generate angiotensin I from excess substrate angiotensinogen, measured by an in-house radioimmunoassay, as described previously.[128]

The mean of the three baseline BP measurements were calculated and compared with the results of the ABPM.

Informed Consent and Ethics Committee

Informed consent was obtained after oral and written information had been given. The ethics committee of the Erasmus MC – Sophia Children's Hospital, University Medical Center Rotterdam approved the study protocol.

Statistics

Data were imported and analysed by a SPSS 11.0 (Inc, Chicago) software programme. Results are presented as mean, standard deviation (SD) and 95% confidence intervals (95%CI). Multivariate linear regression analyses adjusted for gender were performed to analyze the differences in BP and renin concentration between the three groups. Differences between SGA and AGA participants were accounted as differences due to IUGR, and differences between AGA and controls were accounted as differences due to GA. We also compared the BP values obtained by initial screening and 24-hour BP monitoring, with Bland and Altman plots and 95% limits of agreement.

The relation between IUGR and BP was analyzed with the regression model with 'unstandardized residuals', as we described recently.[97] This because birth weight (SDS) or IUGR is related to adult weight (SDS), and adult weight is related to BP (causal pathway). In this model the relation between BW-sds and adult weight (SDS) is calculated. The residual for adult weight, expressing the increase in adult weight upon a given birth weight (SDS), is entered in the regression model.

The prevalence of hypertension within the groups and the relative risks (RR) with 95%CI were calculated between groups. Differences were considered statistical significant at the level of 0.05.

RESULTS

Eighty-two individuals participated in the study (23 SGA, 29 AGA and 30 controls). Of 23 SGA individuals, 2 individuals were excluded from data analysis: 1 because of very frequent premature heartbeats leading to unreliable BP measurement, and another as a result of an unrelated allergic episode prior to the ABPM measurement. Baseline characteristics of the remaining 80 individuals in the 3 groups are shown in Table 1. Weight and height were statistically-significantly lower in SGA individuals compared with AGA and controls. BMI at age 20 did not differ between the three groups (SGA 21.6 kg/m^2, AGA 22.1 kg/m^2 and controls 22.9 kg/m^2). Maternal hypertension during pregnancy and alterations on cardiotocographic measurement were more common in SGA compared with AGA subjects, but other prenatal, perinatal and postnatal parameters were not. (Table 2) No association between these parameters and adult blood pressure was present in these preterm born individuals.

Birth weight (g), BW-sds and GA were not different between men and women. Men had greater body height (mean difference 13.4 cm 95%CI 10.0;16.9) and higher body weight (mean difference 12.3 kg 95%CI 7.7;17.0), but equal BMI (mean difference 0.6 kg/m^2 95%CI –0.7;1.9) compared with women.

Ambulant Blood Pressure Monitor

Daytime SBP was 6.6 mmHg (95%CI 3.2;10.1) higher in men (125.6 mmHg) than in women (119.0 mmHg). Similar differences were found for overall, night time and baseline SBP. DBP and MAP were not statistically-significantly different between men and women. The daytime heart frequency was 5.8 bpm (95%CI 2.2;9.3) lower in men than in women, with similar differences for overall, night time and baseline HF. In women the mean nocturnal dip of MAP was 15.0%, and in men 12.1% (mean difference 2.9% with 95%CI 0.5;5.3 and p value 0.02). Further analyses comparing BP values between the three groups were adjusted for gender.

Prematurity Overall, daytime and baseline SBP was higher in AGA individuals compared with controls (Table 3). At baseline SBP was 8.4 mmHg higher (95%CI 4.1;12.7, p-value < 0.001), but baseline DBP did not differ. Also, ambulant daytime SBP was 4.2 mmHg higher in AGA individuals compared with controls (95%CI 0.4; 8.0, p-value: 0.03). Night time SBP did not differ statistically-significantly between AGA and controls (AGA 3.6 mmHg higher with 95%CI -0.9; 8.1, p-value: 0.12). The nocturnal SBP dip did not differ between AGA and controls. Baseline MAP was higher in AGA individuals compared with controls. Ambulatory DBP, HF, and MAP did not differ between the controls and AGA.

IUGR No significant differences in SBP, DBP, MAP and HF were present between the SGA and AGA group, both during day and night. This implies that IUGR in individuals

Table 1: Subject baseline characteristics.

	SGA N = 21	AGA vs SGA Mean difference (95%CI)	AGA N = 29	Controls vs AGA Mean difference (95%CI)	Controls N=30	Controls vs SGA Mean difference (95%CI)
Age (yrs)	20.7 (0.3)	0.0 (-0.2;0.2)	20.7 (0.4)	0.0 (-0.3;0.3)	20.7 (0.8)	0.0 (-0.4;0.4)
Men (% (number)) *	38.1 (8)	-0.2 (p value 0.9)	37.9 (11)	7.8 (p value 0.5)	46.7 (14)	8.6 (p value 0.5)
BW (gram)	858.1 (131.6)	631 (507;754) ‡	1489.0 (257.5)	2134 (1938;2348)‡	3632.0 (488.7)	2774 (2554;2994) ‡
GA (weeks)	30.6 (1.1)	-1.1 (-1.8;-0.4) †	29.5 (1.4)	10.7 (10.0;11.4)‡	40.2 (1.3)	9.6 (8.9;10.3) ‡
BW (SDS)	-2.2 (0.3)	2.9 (2.7;3.1) ‡	0.7 (0.5)	-0.4 (-0.8;0.0)	0.3 (1.0)	2.5 (2.1;3.0) ‡
Body height (cm)	167.6 (11.5)	6.6 (1.2;12.1) †	174.3 (7.7)	2.0 (-2.7;6.6)	176.2 (10.0)	8.8 (2.3;14.4) †
Body weight (kg)	60.7 (9.1)	6.7 (0.4;13.0) †	67.4 (12.0)	4.0 (-2.2;10.2)	71.4 (11.7)	10.7 (4.6;16.9) ‡
Body weight (SDS)	-1.1 (1.3)	0.9 (0.2;1.5) †	-0.2 (1.0)	0.4 (-0.2;1.0)	0.2 (1.2)	-1.2 (-2.0;-0.5) ‡
BMI (kg/m^2)	21.6 (2.6)	0.5 (-1.1;2.1)	22.1 (2.8)	0.8 (-0.6;2.3)	22.9 (2.8)	1.3 (-0.2;2.9)

Data expressed by mean ± standard deviation, except when notified differently. SGA: Small for GA; AGA: Appropriate for GA; BW: birth weight; GA: GA; BW (SDS): birth weight adjusted for GA expressed by standard deviation scores. P-values based on linear regression analysis between groups. * P-value based on χ^2-test. † P-value < 0.05, ‡ P-value < 0.001

Table 2: Presence of obstetric and perinatal parameters in the premature born individuals (SGA and AGA).

Obstetric and Perinatal parameters	SGA (21)		AGA (29)	
	Number	%	Number	%
Hypertension during pregnancy	13	62 %	2	7 %
Diabetes Gravidarum	1	5 %	2	7 %
Blood group Antagonism	0	0%	0	0 %
Pre-existing maternal disease	2	10 %	1	3 %
Premature rupture of membranes	2	10 %	8	28 %
Amnionitis	0	0 %	3	10 %
Cardiotocographic deterioration	15 (3 unknown)	71 %	4 (7 unknown)	14 %
Use of corticosteroids	2	10 %	8	28 %
Unfortunate Apgar score 5 minutes	1 (2 unknown)	5 %	1 (2 unknown)	3 %
Infant respiratory distress syndrome	5	24 %	16	55 %
Sepsis	8	38 %	13	45 %
Convulsions	0	0%	0	0%

born very preterm does not influence young adult BP. Crude mean values and mean differences adjusted for gender are presented in Table 3.

Both IUGR and prematurity Baseline SBP was 7.1 mmHg higher in SGA individuals than in controls (95%CI 2.0;12.0, p-value:0.007). Daytime SBP was 3.6 mmHg higher in SGA individuals than in controls, but the difference did not reach significance (95%CI -0.9;8.0, p-value: 0.111). Night time SBP, and both day and night DBP, MAP and HF did not differ between SGA individuals and controls.

Birth weight adjusted for GA (BW-sds) was not related to adult daytime SBP, adjusted for gender and residual adult weight.[97] Residual adult weight remained related to daytime SBP. BMI was not related to adult daytime SBP. BW-sds was positively related to adult weight (sds) with a regression coefficient of 0.389 SD/SD (95%CI 0.223;3.079, p-value < 0.001). Adult weight (sds) was positively related to adult daytime SBP (β = 1.5 mmHg/SD, 95%CI 0.1;2.8 p-value 0.03) adjusted for gender.

Both actual birth weight (grams) and GA (GA: weeks) were negatively related to adult SBP. The regression coefficient was –0.14 mmHg per 100 gram increase in BW (95%CI –0.3;0.0) and –0.39 mmHg / week increase of gestation (95%CI –0.72;-0.06). When only preterm individuals (SGA and AGA) were included in this analysis, the point estimate became -0.09 mmHg per 100 grams BW and -0.56 mmHg per week GA, but significance disappeared in both comparisons.

Table 3: Blood pressure and renin concentration

	SGA N = 21	SGA vs AGA Mean difference (95% CI) Adjusted for gender		AGA N = 29	AGA vs Controls Mean difference (95% CI) Adjusted for gender		Controls N=30	SGA vs Controls Mean difference (95% CI) Adjusted for gender	
Baseline SBP (mmHg)	124.1 (10.9)	1.3	(-4.1;6.6)	125.4 (9.9)	-8.4	(-12.7;-4.1) ‡	117.7 (8.2)	-7.1	(-12.1;2.0) ‡
Baseline DBP (mmHg)	70.0 (9.8)	0.0	(-5.1;5.0)	70.0 (7.8)	-1.9	(-5.8;2.0)	68.0 (7.1)	-1.8	(-6.5;3.0)
Daytime SBP (mmHg)	122.7 (8.7)	0.4	(-3.9;4.8)	123.1 (8.5)	-4.2	(-8.0;-0.4) †	119.6 (7.6)	-3.6	(-8.0;0.9)
Night time SBP (mmHg)	107.9 (8.3)	3.0	(-1.7;7.7)	110.9 (9.1)	-3.6	(-8.1;0.9)	107.9 (9.4)	-0.3	(-5.4;4.7)
Nocturnal SBP dip (%)	12.0 (5.3)	-2.0	(-4.8;0.7)	9.9 (4.4)	-0.1	(-2.4;2.2)	9.8 (9.5)	-2.2	(-5.0;0.6)
Daytime DBP (mmHg)	73.0 (8.0)	-0.8	(-4.6;2.9)	72.2 (5.1)	-0.3	(-3.3;2.6)	71.7 (6.0)	-1.0	(-4.9;2.9)
Night time DBP (mmHg)	58.1 (7.3)	1.6	(-2.1;5.3)	59.6 (5.6)	-1.0	(-4.3;2.4)	58.8 (7.1)	0.8	(-3.4;4.9)
Nocturnal DBP dip (%)	20.3 (7.6)	-3.0	(-7.0;1.0)	17.3 (6.4)	1.1	(-2.2;4.4)	18.1 (6.5)	-2.0	(-6.0;2.0)
Renin * (ng Al/ml/h)	1.77 (0.78)	0.42	(-0.08;0.92)	2.18 (0.93)	0.05	(-0.48;0.57)	2.26 (1.08)	0.48	(-0.09;1.05)

Data expressed by crude mean ± standard deviation. SGA: Small for GA; AGA: Appropriate for GA; SBP: systolic blood pressure; DBP: diastolic blood pressure. * Renin concentration in 20 SGA individuals. Baseline values were measured at an initial screening with Hewlett Packard device. Daytime and night time values were measured with an ambulatory blood pressure device (Spacelabs™) during 24 hours. † P-value < 0.05, ‡ P-value < 0.01

Table 4: Prevalence of hypertension

	SGA		AGA		Controls	
	N	(%)	N	(%)	N	(%)
Normal BP	6	(28.6)	8	(27.6)	16	(53.3)
Prehypertensive stage	14	(66.7)	19	(65.5)	14	(46.7)
Hypertension stage 1	1	(4.8)	2	(6.9)	0	(0)
Total	21	(100)	29	(100)	30	(100)

JNC VII criteria for hypertension[95]:
Normal BP: SBP < 120 mmHg and DBP < 80 mmHg
Prehypertensive stage: SBP 120-139 mmHg or DBP 80-89 mmHg
Hypertension stage 1: SBP 140-159 mmHg or DBP 90-99 mmHg
(No cases of hypertension stage 2: DBP >160 mmHg or DBP >100 mmHg)

Hypertension prevalence

Table 4 shows the prevalence rates of hypertension within the three groups. The distribution of normal, prehypertension and hypertension stage 1 was equal (χ^2 0.006, p-value 0.94) between SGA and AGA individuals. The distribution of AGA differed significantly from controls (χ^2 4.051, p-value 0.04). Elevated BP (either prehypertensive stage or hypertension) was 1.6 (95%CI:1.0;2.4, p-value =0.05) times more common in AGA individuals, and 1.5 (95%CI:1.0;2.4, p-value =0.08) times more common in SGA compared with controls. No difference in prevalence of elevated BP was present between SGA and AGA individuals.

Renin concentration

Renin concentration (ng AI/ml per hour) was available in 79 of the 80 individuals (Table 3). Renin concentration was not significantly higher in men than in women (mean difference: 0.33 ng AI/ml/h, 95%CI –0.1:0.7). In SGA individuals renin concentration was 0.42 lower compared with AGA individuals (95%CI –0.08;0.92, p-value: 0.10) and 0.48 lower compared with controls (95%CI -0.09;1.05, p-value: 0.10). No relation between renin concentration and systolic or DBP was found. In a linear regression analysis renin concentration was not related to birth weight as a continuous variable (both SDS and g) or to GA.

Comparison of baseline BP measurement with ABPM

The baseline SBP overestimated the daytime systolic ABPM with 0.5 mmHg (95% limits of agreement of –14.7 to 15.7). The baseline DBP underestimated the diastolic ABPM with -2.9 mmHg (95% limits of agreement: -15.4;9.5).

DISCUSSION

We found that SBP was increased at the age of 20 years in individuals born very prematurely (< 32 weeks) compared with full term born individuals of the same age. Second, IUGR did not alter this effect on SBP within these very preterm individuals. This suggests that prematurity is a predisposing risk factor for the development of increased SBP and no additional effect of IUGR within very preterm born subject is present. No differences in nocturnal BP or nocturnal dip were present in our study.

The results are in agreement with our previous study [133] showing a similar mean SBP of 123 mmHg, and a high prevalence of hypertension (10%) in individuals who were born very preterm recruited from the same cohort. In this previous study the mean of three BP measurements at one initial screening visit were used and controls were lacking. Therefore, 24-hour BP measurements in preterms and controls were used in the present study. Both studies suggest that IUGR within individuals who are born prematurely does not predispose to an increased BP, but that prematurity is the important factor. Likewise, Irving et al. has shown that BP was increased at the age of 24 years in individuals who were born prematurely (mean GA 33.4 weeks) and no effect of IUGR within this group was shown.[68] A Swedish study in 165,136 men also showed an inverse association between GA (GA), ranging from 35 - 44 weeks, and SBP.[5]

In contrast, Singhal et al. did not find an increased risk of vascular disease at the age of 15 years within 216 preterms (mean GA of 31 weeks) compared with at term born individuals of the same age.[69] Also a large cohort study in Brazil could not detect a difference in BP values between the 15-year-old individuals born with a GA below and over 37 weeks.[134] Only birth weight adjusted for GA (IUGR) revealed a relation to adolescent BP in this study. One possible explanation for the contrast of results in these adolescent individuals may be that the relation between early determinants of increased BP may be obscured during adolescence as also described by Barker et al. [12;111]

Siewert-Delle suggested that the effect of GA may be even stronger in the lower ranges of gestation (GA 30-38 weeks).[65] The effect of IUGR may therefore be concealed in our study population. In our study the regression coefficient of GA to adult SBP became slightly stronger when only the very preterm individuals were included, but significance disappeared. This may be due to a decreased number of individuals. However, in a larger sample the very preterm individuals of our cohort no relation between GA was found.[133] Therefore, our data suggest that the relation between GA and adult SBP decreases in the lowest ranges of gestation.

Besides the increased BP values, we also found that the prevalence of prehypertensive stage was 1.5-1.6 times higher in preterms compared with controls. Individuals with prehypertensive BP are known to be at risk to develop hypertension in

later life.[95] High prevalence of prehypertensive stage in our study population (66% in preterms and 45% in controls), suggest that screening at young adult age would possibly be effective to trace individuals at risk of adult hypertension. Early recognition of individuals at risk may help to prevent the development of hypertension and treatment can be introduced early.

No relation between IUGR and prematurity and renin concentration was found. These findings are in contrast with the findings of other studies, showing altered RAS in IUGR individuals.[58;59] Therefore, no contribution to this possible pathophysiological mechanism of the RAS can be given with our data. The absence of the relation between renin and BP may be due to small number of individuals in our study.

Accurate measurements of BP, like ambulant 24-hour BP monitoring, are hard to obtain in large epidemiological studies. Therefore, BP measurements in these studies are often limited to initial screenings. In our study we were able to compare BP when measured by only one initial screening, or by 24 hour ambulant BP monitoring. The mean systematic error in SBP and DBP after an initial screening compared with daytime SBP and DBP were small (0.5 mmHg and –2.9 mmHg respectively). This means that the mean values of both SBP and DBP in epidemiological studies measured on an initial screening will not deviate much from ABPM values. Results of studies based on initial screening BP values are therefore reasonably comparable to studies using ABPM values. However, the limits of agreement were high. This means that individual BP measurements based on an initial screening, may over- or underestimate the ABPM BP with 15.2 mmHg for SBP and 12.5 mmHg for DBP. Thus BP measurement on an initial screening for individual purposes are not recommended, to prevent erroneous diagnosis and inappropriate management, as also described previously.[135]

The strength of our study was that perinatal parameters in our very preterm individuals were obtained prospectively. The retrospectively obtained data on birth weight and GA in controls may have introduced recall bias. Most importantly, controls were only included when born at term (> 37 weeks) and normal birth weight (> 2500 grams). If birth weight or GA was given by mother's recall, we assumed that the mother did recall prematurity or low birth weight. Individuals providing uncertain data on birth weight or GA were not included in the study. Therefore, the chance of recall bias is low.

Second, we were able to evaluate the effect of prematurity separately from IUGR within the very preterm born individuals. The results indicate that very preterm birth affects young adult SBP, but that IUGR in very preterm individuals do not attenuate this effect.

Reliable prevalence rates of hypertension (stage 1 and 2) and relative risks cannot be given, as the number of individuals in our study was small. Much larger samples

are needed to confirm our findings. Unfortunately, in our cohort no larger sample of very preterm SGA individuals could be included, as these were not available within the cohort.

In conclusion, our results support the hypothesis that very premature birth increases both the mean SBP in young adults and the risk of hypertension. In our study, IUGR did not contribute to the increased SBP. Systematic monitoring of the BP in young adults born preterm may identify individuals at risk for development of hypertension. In addition, such follow-up program may reveal the clinical significance of our findings.

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Chapter 4.3

Decreased renal blood flow at young adult age after intra uterine growth retardation and very premature birth

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ABSTRACT

Background: Preterms and intra-uterine growth retardated (IUGR) subjects are presumed to have an increased risk to develop renal disease at adult age. Decreased renal function may already be present at young adult age.

Methods: We included 29 appropriate, 23 small for gestational age (AGA, SGA) very premature individuals (gestational age < 32 weeks) and 30 AGA and full term born controls (37-42 weeks) at age 20. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured by Inulin and ρ-amminohippuric acid clearances (ml/min/1.73m^2) before and after renal stimulation with low dose dopamine infusion (2 µg/kg/min) and oral amino-acid intake. Also, we assessed albumin to creatinine ratio (ACR: mg/mmol) in three urine samples and sonographicly estimated renal size.

Results: At baseline GFR did not differ between the three groups (SGA 107, AGA 116 and Controls 112 ml/min/1.73m^2), but ERPF was significantly lower in SGA compared with controls (515 vs. 586 ml/min/1.73m^2). After renal stimulation GFR and ERPF increased significantly, but the GFR increase tended to be lower in SGA subjects compared with controls (12 and 20 ml/min/1.73m^2). The mean value of ACR was significantly higher in SGA individuals compared with controls (p-value: 0.03). No significant difference between SGA versus AGA, and AGA versus controls was found. Renal size was significantly associated to renal function (p value < 0.001).

Conclusions: Our findings support the hypothesis that preterm birth in combination with IUGR contributes to abnormal renal function at young adult age.

INTRODUCTION

The associations between low birth weight and adult hypertension, the insulin resistance syndrome, type II diabetes and cardiovascular events have been well described.[13;136] It was hypothesized that unfavourable fetal and neonatal conditions lead to permanent alterations in organs and organ systems, resulting in an increased risk of disease. [137]

In intrauterine growth retardated (IUGR) individuals a decreased nephrogenesis is suggested to be responsible for a decrease in renal function at long-term.[41] Animal studies have shown a decreased nephron number and an impaired renal function in low birth weight offspring.[129;138] Also in humans a decrease in nephron number in low birth weight subjects has been found.[117;139] However, prospective follow-up studies in low birth weight individuals evaluating the renal function in adult life are limited and inconclusive.

Recently, it has been shown that nephrogenesis ceases after very premature birth suggesting limited nephron number in preterms throughout life.[63] As a strong relation between nephron number, renal size and albuminuria was shown in individuals at risk of renal diseases, very preterm subjects may be at risk to develop microalbuminuria and renal disease in adult life.[34;45] Recently, we have found that IUGR affects creatinine clearance, microalbumin concentration in spot urines, and systolic blood pressure at the age of 19 years within subjects born very preterm [132;133]. The effect of prematurity itself was not studied.

We describe the effect of prematurity, IUGR and the combination of prematurity and IUGR on microalbuminuria, glomerular filtration rate (GFR), effective renal plasma flow (ERPF) and renal functional reserve capacity (RFRC) at the age of 20 years.

METHODS

Study population

Three groups of young adults were recruited to participate in our study: 1. Individuals born very premature (< 32 weeks) and small for gestational age (SGA), 2. Individuals born very premature, and appropriate for gestational age (AGA), 3. Controls born full term (37-42 weeks) and appropriate for gestational age. The individuals who were born very premature were recruited from the participants of the POPS 19 study (N=422) [132]; A follow-up study of a Dutch birth cohort (all born in 1983) consisting of 94% of all live born individuals in the Netherlands with a gestational age < 32 weeks and/or a birth weight < 1500 grams (Project on Prematures and Small for gestational age infants (POPS)). Birth weight and gestational age of these

individuals were derived from the POPS database (TNO Quality of Life, Leiden, The Netherlands). Birth weight was converted to standard deviation scores (SDS) adjusted for gestational age using Swedish reference standards [93] and considered as a measure of IUGR. Recruitment of SGA individuals started with the lowest birth weight adjusted for gestational age (≤ -2 SDS: N=29) and AGA individuals with the highest birth weight adjusted for gestational age (0-2 SDS: N=205). Controls were born between January 1st 1982 and December 31st 1984 and recruited by advertisement. Vegetarians were excluded from the study.

Data obtainment

Patient characteristics Adult weight, height and body mass index (BMI) were obtained and converted in standard deviation scores [94]. Data on birth weight and gestational age of the prematurely born individuals were derived from the POPS database. Data on birth weight and gestational age of the controls was mainly obtained from birth records, or when unavailable by mother's recall.

Baseline renal function Participants were instructed to have a light, fructose free breakfast. Baseline serum creatinine, urea, sodium and potassium concentrations were obtained. Baseline GFR and ERPF were estimated by the mean of three 30 minutes clearance periods with respectively Inulin (Inutest 25%, Fresenius, Austria) and ρ-aminohippuric acid (PAH: sodium aminohippurate 20%, MSD). A single bolus injection of 45 mg/kg Inulin and 8 mg/kg PAH preceded the continuous infusion of 42 mg/min Inulin and 20 mg/min PAH. Participants were instructed to void 45 minutes after the bolus injection (t=0) and at t=30, t=60 and t=90 minutes. Blood samples were taken, from the opposite arm where medication was infused, in the middle of each clearance period at t=15, t=45 and t=75 minutes.

Stimulated renal function After baseline renal function test, the participants were given a protein-rich lunch (containing 130 g beef-steak).[124;125] At least sixty minutes after starting lunch and at least forty minutes after finishing lunch, a low dose dopamin infusion was started at a rate of 2 μg/kg/min.[124] After five minutes three new clearance periods were started: Voiding at t=150, t=180, t=210, and t=240 and blood samples at t=165, t=195, and t=235. Emptying of the bladder was monitored estimating the residual volume in the bladder with a bladder scan directly after voiding.

Calculation of GFR, and ERPF GFR and ERPF adjusted for body surface area (BSA) were calculated using the equation: $U_{Inul/PAH}$ * Volume (ml) * 1.73 / $P_{Inul/PAH}$ * duration (min) * BSA, in which $U_{Inul/PAH}$ and $P_{Inul/PAH}$ reflects the concentration (mg%) of Inulin or PAH in the urine and plasma respectively, and BSA the √ (height (m) * weight (kg) / 3600). The filtration fraction (FF) was calculated by dividing GFR by ERPF (*100%). The mean of three measurements before and after renal stimulation was used in

the analysis. To monitor systemic effects of dopamin infusion, we obtained systolic and diastolic blood pressure (SBP, DBP) using a calibrated automatic blood pressure device (Hewlett Packard).

Microalbuminuria Three morning urines were obtained at three consecutive days prior to the renal function test to measure albumin to creatinine ratio (ACR: mg/mmol). An ACR between 1.1 and 2.2 was considered as high normal, and > 2.2 mg/mmol as microalbuminuria.[87;140]

Renal ultrasound Measures of maximal bipolar kidney length and renal volume ((π / 6) * length (cm) * width (cm) * thickness (cm)) were obtained by renal ultrasound. Relative renal length and volume were calculated dividing renal length by body height, and renal volume by BSA respectively. The mean of left and right kidney size was used in the analysis.

Laboratory assays

Laboratory analyses were determined on a Hitachi 917 (Roche Diagnostics). Electrolytes were measured by using Ion Selective Electrodes. Enzymatic assays were used for the determination of creatinine (CREA Plus®) and urea in urine and serum. For the determination of microalbumin in urine an immunoturbidimetric assay was used. Inulin and PAH concentrations were measured using the automatic Anthrone and Bratton-Marshall technique.[126;127]

Informed Consent and Ethics Committee

Informed consent was obtained after oral and written information had been given. The ethics committee of the Erasmus MC – Sophia Children's Hospital, University Medical Center Rotterdam approved the study protocol.

Statistics

Results are presented as mean, standard deviation (SD) and 95% confidence intervals (95%CI). Linear regression analyses were performed to analyse the differences between the three groups in GFR, ERPF, FF, blood pressure, and diuresis before and after renal stimulation. Change in GFR, ERPF, and FF were adjusted for the baseline value. To evaluate the change in GFR, ERPF and FF after renal stimulation a paired *t*-test was used. Statistical significance was considered at the level of 5%.

Table 1: Subject baseline characteristics.

	SGA N = 23		AGA vs SGA Mean difference (95%CI)		AGA N = 29		Controls vs AGA Mean difference (95%CI)		Controls N=30		Controls vs SGA Mean difference (95%CI)	
Age (yrs)	20.7	(0.3)	0.0	(-0.2:0.2)	20.7	(0.4)	0.0	(-0.3;0.3)	20.7	(0.8)	0.0	(-0.4;0.4)
Males (% number)) *	43.5	(10)	- 5.6	(p value 0.7)	37.9	(11)	7.8	(p value 0.5)	46.7	(14)	3.2	(p value 0.8)
BW (gram)	858.7	(125.7)	630.3	(513;748) ‡	1489.0	(257.5)	2134	(1938;2348)‡	3632.0	(488.7)	2773	(2563;2983) ‡
GA (weeks)	30.6	(1.0)	-1.1	(-1.8;-0.4) †	29.5	(1.4)	10.7	(10.0;11.4)‡	40.2	(1.3)	9.6	(8.9;10.2) ‡
BW-sds	-2.2	(0.3)	2.9	(2.7:3.1) ‡	0.7	(0.5)	-0.4	(-0.8;0.0)	0.3	(1.0)	2.5	(2.1;3.0) ‡
Body height (cm)	167.8	(11.1)	6.5	(1.2:11.7) †	174.3	(7.7)	2.0	(-2.7;6.6)	176.2	(10.0)	8.5	(2.6;14.3) ‡
Body weight (kg)	60.9	(8.9)	6.5	(0.4;12.5) †	67.4	(12.0)	4.0	(-2.2;10.2)	71.4	(11.7)	10.5	(4.6;16.4) ‡
BMI (kg/m^2)	21.6	(2.5)	0.5	(-1.0:2.0)	22.1	(2.8)	0.8	(-0.6;2.3)	22.9	(2.8)	1.3	(-0.2;2.8) §

Data expressed by mean ± standard deviation, except when notified differently.

SGA: Small for gestational age; AGA: Appropriate for gestational age; BW: birth weight; GA: gestational age; BW-sds: birth weight adjusted for gestational age expressed by standard deviation scores.

P-values based on lineair regression analysis between groups.

* P-value based on χ^2-test.

§ P value < 0.10, † P-value < 0.05, ‡ P-value < 0.01

RESULTS

Eighty-two individuals participated in the study (23 SGA, 29 AGA and 30 controls). In controls, data on birth weight and gestational age were subtracted from written birth certificates or medical records in 18 individuals (60%) and from mothers' recall in 12 individuals (40%).

In three participants (1 person in each group) renal function test was not finished due to adverse reaction to the study medication. Blood pressure during the renal function test was measured in 78 of the remaining 79 subjects, (due to very frequent premature heartbeats leading to unreliable blood pressure values in 1 person). Data on microalbuminuria was present in all participants.

Baseline characteristics of all 82 subjects are shown in table 1. Weight and height at age 20 were significantly lower in SGA subjects compared with AGA and controls, but BMI was equal. The distribution of males and females was equal between groups.

Renal function test

Table 2 shows the mean (SD) values of GFR (Figure 1A), ERPF (Figure 1B), and FF in the three groups before and after renal stimulation, and the mean (SD) ACR and logarithmic values of ACR measured in three morning urines. No significant differences in renal functions were present between males and females (data not shown).

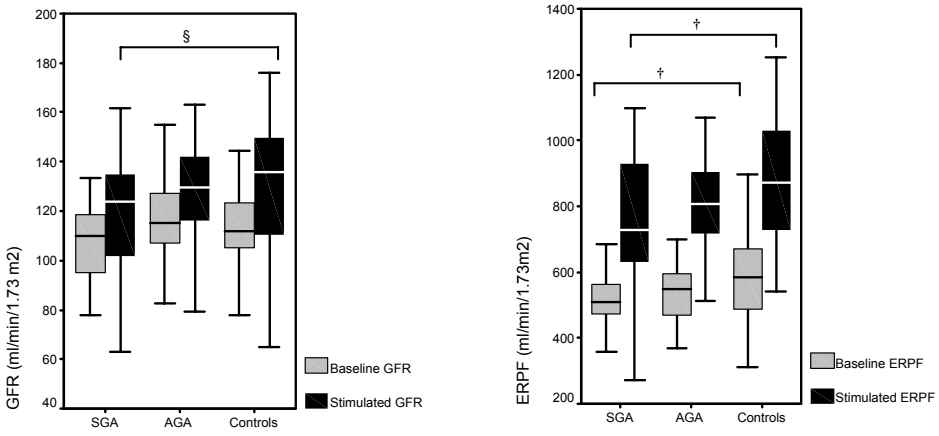

Figure 1: Mean Glomerular filtration rate (GFR) (Figure 1A) and Effective renal plasma flow (ERPF) (Figure 1B) at baseline and after renal stimulation in the three study groups. § P-value < 0.10 and † P-value < 0.05.

Table 2: Renal function in three study groups.

	SGA N = 22 * Mean (SD)		AGA vs SGA Mean difference (95% CI)		AGA N = 28 * Mean (SD)		Controls vs AGA Mean difference (95% CI)		Controls N=29 * Mean (SD)		Controls vs SGA Mean difference (95% CI)	
At baseline												
GFR (ml/min/1.73m^2)	107.1	(15.0)	9.0	(-2.5;20.6)	116.1	(23.3)	-4.5	(-16.5;7.5)	111.6	(21.9)	4.5	(-6.4;15.5)
ERPF (ml/min/1.73m^2)	514.8	(69.7)	19.0	(-42.2;80.2)	533.9	(128.5)	52.3	(-19.3;123.9)	586.1	(140.7)	71.3	(5.5;137.1) †
FF (%)	21.0	(2.5)	1.1	(-0.2;2.5) §	22.1	(2.2)	-2.5	(-4.1;-0.9) ‡	19.6	(3.6)	1.3	(-0.5;3.1)
After renal stimulation												
GFR (ml/min/1.73m^2)	118.7	(23.5)	11.4	(-2.7;25.5)	130.1	(25.5)	1.4	(-12.3;15.0)	131.5	(26.0)	12.7	(-1.4;26.9) §
ERPF (ml/min/1.73m^2)	747.9	(201.6)	60.2	(-48.9;169.2)	808.1	(181.3)	70.0	(-30.3;170.2)	878.1	(195.7)	130.1	(17.5;242.8) †
FF (%)	16.5	(3.4)	0.1	(-1.7;1.6)	16.4	(2.3)	-1.1	(-2.6;0.3)	15.3	(3.0)	1.2	(-0.6;3.0)
Change due to stimulation												
GFR (ml/min/1.73m^2)	11.6	(16.0)	2.3	(-7.3;12.0)	13.9	(17.5)	5.9	(-3.5;15.3)	19.8	(18.0)	8.2	(-1.6;17.9) §
ERPF (ml/min/1.73m^2)	233.1	(164.6)	41.1	(-36.1;118.4)	274.2	(106.2)	17.7	(-51.3;86.7)	291.9	(149.3)	58.8	(-29.8;147.5)
FF (%)	-4.5	(2.4)	-1.2	(-2.4;0.0) †	-5.7	(1.8)	1.3	(0.0;2.6) †	-4.4	(2.9)	-0.1	(-1.6;1.4)
log ACR (mg/mmol)	-0.43	(0.53)	-0.13	(-0.36;0.09)	-0.56	(0.27)	-0.11	(-0.23;0.02) §	-0.67	(0.21)	-0.24	(-0.45;-0.03) †
Geometric mean ACR	0.37				0.28				0.22			

Data expressed by mean ± standard deviation, mean difference and 95% CI.

SGA: Small for gestational age; AGA: Appropriate for gestational age; GFR: glomerular filtration rate (ml/min/1.73m^2); ERPF: Effective renal plasma flow (ml/min/1.73m^2); FF: Filtration fraction (%); ACR: albumin creatinine ratio (mg/mmol).

* Total number of subjects in ACR was 82 (23 SGA + 29 AGA + 30 Controls).

§ P value < 0.10, † P-value < 0.05, ‡ P-value < 0.01

Baseline measurements At baseline GFR did not differ between the three groups (107, 116 and 112 ml/min/1.73m^2 in SGA, AGA and controls respectively). ERPF at baseline was significantly lower in SGA (515 ml/min/1.73m^2) compared with controls (586 ml/min/1.73m^2). The ERPF in AGA individuals at baseline was with 534 ml/min/1.73m^2 not statistically different from both SGA and controls. FF was 1.3% (95%CI:-0.5;3.1) higher in SGA and 2.5% (95%CI:0.9;4.1) higher in AGA compared with controls. SBP during the renal function test was significantly higher in both SGA and AGA subjects compared with controls. DBP was not different between the three groups.

Change due to stimulation GFR, ERPF, and diuresis increased significantly, and FF dropped in all groups (paired t-test). SBP did not change, but DBP decreased with 4.2 mmHg (95%CI:2.7;5.7). The increase in GFR due to renal stimulation (adjusted for baseline GFR) tended to be smaller in SGA subjects compared with AGA and to controls (crude GFR increase 11.6, 13.9 and 19.8 ml/min/1.73m^2 respectively, p-value 0.09). The increase in ERPF was not statistically different between the three groups.

Measurements after stimulation After stimulation the mean GFR in SGA was 119 ml/min/1.73m^2, compared with 130 and 132 ml/min/1.73m^2 in AGA and controls respectively (mean difference SGA vs. controls: 12.7 (95%CI:-1.4;26.9)). ERPF was 130 ml/min/1.73m^2 lower in SGA (95%CI:17.5;242.8) and 70 ml/min/1.73m^2 lower in AGA (95%CI:-30;170) than controls.

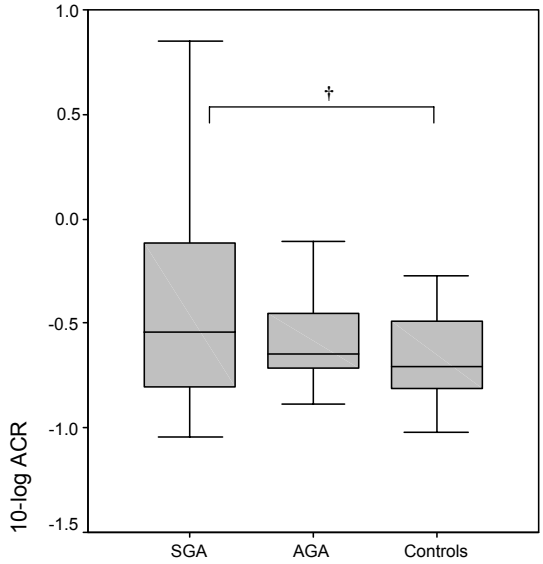

Figure 2: Mean albumin creatinine ratio (ACR) (10-logarithm transformed) in the three study groups. † P-value < 0.05.

Table 3: Regression coefficients renal size predicting renal function outcomes.

	Baseline GFR		Stimulated GFR		Baseline ERPF		Stimulated ERPF	
	β	(95%CI)	β	(95%CI)	β	(95%CI)	β	(95%CI)
Mean bipolar kidney length (cm)	9.2	(4.0;14.4) ‡	12.4	(6.1;18.7) ‡	60.6	(29.8;91.4) ‡	102.7	(54.4;151.0) ‡
Mean relative kidney length (cm/m)	18.0	(7.6;28.3) ‡	23.1	(10.3;35.8) ‡	127.4	(66.7;188.1) ‡	201.7	(105.1;298.3) ‡
Mean kidney volume (ml)	0.15	(0.03;0.28) †	0.25	(0.10;0.40) ‡	0.85	(0.08;1.62) †	1.61	(0.40;2.82) ‡
Mean relative kidney volume (ml/m^2)	0.27	(-0.02;0.52) *	0.44	(0.12;0.76) ‡	1.37	(-0.24;2.97) *	2.70	(0.17;5.23) †

Regression coefficients and 95%CI of sonographically measured renal size on renal function adjusted for gender.
* p value < 0.10, † p value < 0.05, ‡ p value < 0.001.

Microalbuminuria

Microalbuminuria (ACR > 2.2) was present in 2 SGA subjects (8.7% of all SGA subjects), but not in AGA subjects or controls. High normal values of ACR (1.1-2.2) were present in 2 SGA subjects (8.7%), 1 AGA subject (3.4%) and none of the controls. After logarithmic transformation of ACR (left-sided skewed) ACR was 0.24 (95%CI 0.03;0.45) higher in SGA compared with controls (Table 2 and Figure 2). This corresponds with a mean difference in (geometric) ACR of 1.74 mg/mmol 1.1;2.8)).

Renal ultrasound and renal function

The mean absolute and relative kidney length and volume were all positively associated with baseline and stimulated GFR and ERPF (Table 3). An increase of 1 cm in bipolar kidney length increased baseline GFR with 9.2 ml/min/1.73m^2 (95%CI: 4.0;14.4), stimulated GFR with 23.1 ml/min/1.73m^2 (95%CI: 6.1;18.7), baseline ERPF with 60.6 ml/min/1.73m^2 (95%CI: 29.8;91.4) and stimulated ERPF with 102.7 ml/min/1.73m^2 (95%CI: 54.4;151.0).

DISCUSSION

It has been postulated that progressive renal function loss is characterized at first by single nephron (SN) hyperfiltration, or increased SN-GFR, and increased microalbuminuria and overt albumin leakage afterwards.[141] With this increase in SN-GFR the renal functional reserve capacity (RFRC) is consumed, to maintain normal renal function, and the measured RFRC is decreased.[142] Estimation of the RFRC can be established calculating the difference in GFR before and after afferent and efferent vasodilatation by ingestion of amino-acids and infusion of (low dose) dopamin respectively.[124] The difference in ERPF and FF can help to evaluate whether a potential difference in RFRC is a result of a (combination of) decreased capacity of afferent or efferent vasodilatation.

In healthy individuals maximal efferent vasodilatation (dopamin effect) leads to an increase in both ERPF and GFR, but as intraglomerular pressure decreases, ERPF rises more than GFR, and FF falls. In our study, the mean FF fell in all study groups, suggesting an intact capacity for efferent vasodilatation of both preterm and full term born individuals. The trend for a slightly decreased RFRC in the SGA group would therefore be a result of a decreased capacity of afferent vasodilatation (amino-acid effect). This may be a result of either a vascular smooth muscle relaxing problem, or a persistent afferent vasodilatation at baseline combined with an increased intraglomerular pressure. The higher baseline FF in SGA (n.s.) and AGA individuals ($p<0.05$) compared with controls suggests an increased intraglomerular

pressure at baseline. Therefore, we hypothesize that in the very premature participants afferent renal arteriolae are dilated at baseline, leading to a higher risk of glomerular damage over time. Also in AGA baseline ERPF and increase in GFR seemed lower compared with controls, but no significant differences were observed in our study. It seems that IUGR exacerbates the adverse effect of preterm birth on young adult renal function.

Most likely, a decreased number of nephrons in combination with afferent vasodilatation explains the combination of our findings: decreased ERPF with normal GFR at baseline (higher FF), equal increase in ERPF, and smaller increase in GFR due to renal stimulation in SGA compared with controls. This concurs with the finding that microalbuminuria was increased in prematurely and SGA born individuals.[132]

Several studies demonstrated a decreased number of nephrons after IUGR and premature birth.[63;117;129;138;139] Decreased nephron number would decrease filtration surface area leading to hyperfiltration, glomerular hypertension, glomerular damage, eventually leading to progressive renal disease.[41] The decreased renal length and volume in preterms and the associations between renal size and function further support this hypothesis.[34;45]

Our previous study showed similar small, but significant differences in estimated creatinine clearance and microalbuminuria between SGA and AGA individuals born very premature at the age of 19 years suggesting that the difference in renal function between SGA and controls is not only a result of IUGR, but also of prematurity.[132] Animal models have shown a decreased renal function after IUGR.[46;129;138] In humans, a limited number of studies have compared renal function in preterm or IUGR subjects with at term born subjects.[66;143] A decreased creatinine clearance and disturbed tubular function during childhood (6-12 years of age) was observed in very premature SGA and AGA born subjects (mean BW 845 g and GA 27.6 weeks).[143] Also, in 26-year-old women born prematurely (AGA), unfavourable GFR and ERPF was present, but no significant difference with at term born controls was detected.[66] Both studies did not measure the effect of renal stimulation on renal function.

We hypothesize that nephron number in very preterm subjects is impaired and attenuated by IUGR. This leads to an impaired renal functional reserve capacity and long-term renal function loss. Second, an altered (renal) arterial reactivity due to intra uterine stress may have resulted in both an increase in systemic and glomerular blood pressure leading to an affected long-term renal function. [27;58;59] Systolic blood pressure was significantly higher in both SGA and AGA ex-preterms compared with controls, but renal function was more disturbed in SGA.[66;67;133], suggesting that increased blood pressure precedes renal function loss in very preterm subjects, as also described in experimental studies.[144;145]

We were able to evaluate the effect of prematurity separately from the effect of IUGR on adult renal function. At age 20 only the combination of prematurity and IUGR shows significant differences in renal function, even with limited number of subjects in our study. To confirm our findings, extended renal function tests are recommended in larger epidemiological studies. The fact that our participants are still young, and glomerular function decreases with increase in age, underlines the importance of our findings.[120;122] The combination of afferent vasodilatation and higher systolic blood pressure increases the risk for glomerular damage even more. Follow-up of these very preterm subjects is recommended to detect renal function loss in an early phase.

In conclusion, our study indicates that both prematurity and IUGR influence adult renal function. It is likely that (fetal) kidney development is affected leading to an unfavourable renal function in young adult life. Whether these differences will increase over time, and whether these differences are also present during childhood are important perspectives in this field of research. It is warranted to determine if and when screening for renal function loss in very preterm subjects is recommended.

ACKNOWLEDGEMENTS

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Chapter 4.4

Reduced renal length and volume 20 years after very preterm birth

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ABSTRACT

Background: Intrauterine growth retardation is presumed to be associated with decreased renal size and impaired renal function as a result of impaired kidney development and nephron deficit. We studied whether very preterm birth also affects renal size at young adulthood.

Methods: Fifty-one very premature subjects (< 32 weeks of gestation), either small or appropriate for gestational age (22 SGA and 29 AGA), and 30 full term born controls were included to measure bipolar kidney length and kidney volume sonographicly 20 years after birth. Relative kidney length (kidney length / body height) and volume (kidney volume / body surface area) were calculated.

Results: Both absolute and relative left kidney length and volume were significantly lower (1.0 cm (95%CI 0.2;1.2) and 0.3 cm/m (95%CI 0.0;0.6) respectively) in preterm born participants compared with controls, and more obvious in females. For the right kidney this difference was only present in females. Renal size did not differ between preterm born SGA and AGA participants. In 70.0% of controls left kidney was larger than right, compared to 40.9% in SGA (RR 1.7 95%CI 1.0;3.0) and 48.3% in AGA (RR 1.5 95%CI 0.9;2.3). Renal structural anomalies were only present in 8 premature born participants (Fisher exact P-value = 0.02).

Conclusions: Our data suggest that kidney growth is impaired after preterm birth, especially on the left side, and renal structural anomalies are more frequent compared with full term born controls.

INTRODUCTION

An increasing number of studies show an association between intrauterine growth retardation (IUGR) and underdevelopment of the fetal kidneys, with limited nephron number and decreased renal size.[129;138;146;147] Additionally, data have been published indicating a strong relation between birth weight and renal size, nephron number, glomerular volume, albuminuria and systolic blood pressure.[34;45] These studies underline the hypothesis that subjects liable to IUGR are at risk to renal function loss, due to a nephron deficit, loss of filtration surface area, hyperfiltration, glomerular hypertension and glomerular damage.[36]

It has been described that nephrogenesis continues until 36 weeks of gestation. Therefore, it is likely that very preterm born individuals (gestational age < 32 weeks) have a decreased number of nephrons at birth.[62] In addition, it was demonstrated that nephrogenesis is impaired after preterm birth.[63;64] Moreover, limited postnatal kidney growth until the age of 18 months was also shown in individuals who were born premature.[148] It has not been studied whether adult renal size is also impaired after premature birth compared with full term born individuals, or that renal catch-up growth during childhood is present.

Our study was conducted to compare renal size in young adults born very prematurely (either IUGR or with appropriate birth weight) with renal size in full term born controls.

METHODS

Study population

Three groups of young adults were recruited to participate in our study. The first group was born very premature (< 32 weeks) and small for gestational age (SGA). The second group was born very premature, but appropriate for gestational age (AGA). The third group was born full term and appropriate for gestational age (controls).

The SGA and AGA group were recruited from the database of the Project of Preterms and Small for gestational age infants (POPS cohort). This cohort contains 94% of all subjects born alive in the Netherlands in 1983 with a gestational age < 32 weeks and/or a birth weight < 1500 grams. In 2002-2003 63.8 % of the individuals alive at the age of 19 years participated in the POPS 19 study.[133] In our new study all individuals who participated in the POPS 19 study and born with a gestational age < 32 weeks were eligible for inclusion.

Perinatal parameters (i.e. birth weight and gestational age) were derived from this POPS database (TNO Quality of Life, Leiden, The Netherlands). Birth weight adjusted for gestational age was converted to standard deviation scores (BW-sds), using Swedish and Dutch reference standards, and considered as a measure of IUGR.[93] To convert adult weight in standard deviation scores Dutch reference standards were used.[94]

For the SGA group all individuals with the lowest BW-sds (<-2) were asked to participate in our study (n=26). For the AGA group individuals with a BW-sds between 0 and 2 were asked to participate, starting with the highest BW-sds. As the number of eligible individuals in the AGA group was very large (n=205), the individuals living closer to the hospital were recruited first. The control group, containing students born between January 1st 1982 and December 31st 1984, was recruited in the hospital region by advertisement. Data on birth weight and gestational age in the control group was collected by birth records or, when unavailable, by mother's recall.

Informed Consent and Ethics Committee

Informed consent was obtained after oral and written information had been given. The ethics committee of the Erasmus MC – Sophia Children's Hospital, University Medical Center Rotterdam approved the study protocol.

Renal ultrasound

Participants were invited to the Erasmus MC – Sophia Children's hospital, Rotterdam, the Netherlands. Renal ultrasound was performed by one of the three pediatric radiologists according to a standardized protocol. Renal size was measured in prone position with a Philips ATL HDI 5000 scanner with a C5-2 curved array transducer (Philips BV Nederland, The Netherlands). Measures of maximal bipolar kidney length, width and thickness were obtained from both right and left kidney. Renal width and thickness were measured at the level of the kidney hilum. Renal volume (ml) was calculated using the formula (π / 6) * length (cm) * width (cm) * thickness (cm).[149] Cortical thickness was defined as the distance between the external surface of the kidney and top of the medulla. As absolute renal size is known to be associated with body size, we calculated the relative kidney length (bipolar kidney length (cm) / body height (m)) and relative kidney volume (bipolar kidney length (ml) / body surface area (BSA, m^2)).[123] BSA was calculated using the equation $\sqrt{}$ (body height (m) * weight (kg)) / 3600). If renal or ureterovesical anomalies were noticed they were reported.

Statistics

Results are presented as mean, standard deviation (SD) and 95% confidence intervals (95%CI). Analyses were performed with linear regression analyses to compare groups, and stratified by gender.

Intra- and interobserver variability

Intra- and interobserver variability for the three pediatric radiologists was measured in 7 healthy students (5 females, 2 males). The mean (95%limits of agreement) intra-observer variability for bipolar renal length ranged between 0.0 cm (-1.1;1.1) and 0.1 cm (-1.0;1.2) for the left kidney and 0.1 cm (–0.7;0.9) and 0.4 cm (-0.5;1.4) for the right kidney. The intraclass coefficient (ICC) for intra-observer variability was 0.97 for left bipolar kidney length and 0.88 for right bipolar kidney length. The mean (95% limits of agreement) inter-observer variability for bipolar renal length ranged between 0.0 cm (-1.4;1.5) and 0.2 cm (-1.0;1.5) for the left kidney and 0.1 cm (-0.1;0.3) and 0.2 cm (-0.4;0.8) for the right kidney. The intraclass coefficient (ICC) for inter-observer variability was 0.84 for left bipolar kidney length and 0.86 for right bipolar kidney length. The variabilities of renal volume estimates were larger, but no significant systematic errors within and between observers were present.

RESULTS

Baseline characteristics of study population

Renal ultrasound was performed in 81 individuals: 22 SGA, 29 AGA and 30 controls. Baseline characteristics are shown in Table 1. Birth weight and gestational age were significantly different in the three groups. Current weight and height were lower in SGA individuals compared with AGA and controls, but BMI was equal.

In controls, data on birth weight and gestational age were subtracted from written birth certificates or medical records in 18 individuals (60%) and from mothers' recall in 12 individuals (40%). Perinatal and obstetric parameters of the premature born individuals were known since birth and mentioned in table 2. Hypertension during pregnancy was present in 15 of 22 SGA (68%) and in 2 of 29 AGA (7%) individuals. Perinatal corticosteroids were administered in 2 of 22 SGA (9%) and 8 of 29 AGA (28%) individuals. Parameters on renal function of the postnatal period were not obtained.

Renal size

Absolute kidney size was larger in males compared with females.[123] As expected, left kidneys were larger than right kidneys in the majority of the controls (70%). In

Table 1: Baseline Characteristics.

	SGA N = 22		SGA vs AGA Mean difference (95%CI)		AGA N = 29		AGA vs Controls Mean difference (95%CI)		Controls N=30		SGA vs Controls Mean difference (95%CI)	
Age (yrs)	20.7	(0.2)	0.0	(-0.2:0.2)	20.7	(0.4)	0.0	(-0.3;0.3)	20.7	(0.8)	0.0	(-0.4;0.4)
Males (% number) *	40.9	(9)	-3.0	(p value 0.8)	37.9	(11)	7.8	(p value 0.5)	46.7	(14)	5.8	(p value 0.7)
BW (gram)	860.0	(128.5)	629.0	(509;750) ‡	1489.0	(257.5)	2134	(1938;2348)‡	3632.0	(488.7)	2772	(2557;2987) ‡
GA (weeks)	30.6	(1.0)	-1.1	(-1.9;-0.4) †	29.5	(1.4)	10.7	(10.0;11.4)‡	40.2	(1.3)	9.5	(8.9;10.2) ‡
BW-sds	-2.3	(0.3)	2.9	(2.7:3.1) ‡	0.7	(0.5)	-0.4	(-0.8;0.0)	0.3	(1.0)	2.5	(2.1;3.0) ‡
Body height (cm)	167.2	(11.0)	7.0	(1.8:12.3) ‡	174.3	(7.7)	2.0	(-2.7;6.6)	176.2	(10.0)	9.0	(3.1;14.9) ‡
Body weight (kg)	60.6	(9.0)	6.8	(0.6;12.9) †	67.4	(12.0)	4.0	(-2.2;10.2)	71.4	(11.7)	10.8	(4.8;16.8) ‡
BMI (kg/m^2)	21.7	(2.6)	0.4	(-1.1:2.0)	22.1	(2.8)	0.8	(-0.6;2.3)	22.9	(2.8)	1.3	(-0.3;2.8)

Data expressed by mean ± standard deviation, except when notified differently.

SGA: Small for gestational age; AGA: Appropriate for gestational age; BW: birth weight; GA: gestational age; BW-sds: birth weight adjusted for gestational age expressed by standard deviation scores.

P-values based on linear regression analysis between groups.

* P-value based on χ^2-test.

† P-value < 0.05, ‡ P-value < 0.01

Table 2: Presence of obstetric and perinatal parameters in the premature born individuals (SGA and AGA).

Obstetric and Perinatal parameters	SGA (22)		AGA (29)	
	Number	%	Number	%
Hypertension during pregnancy	15	68 %	2	7 %
Maternal Diabetes during pregnancy	1	5 %	2	7 %
Pre-existing maternal disease	3	14 %	1	3 %
Prolonged rupture of membranes	2	9 %	8	28 %
Amnionitis	0	0 %	3	10 %
Cardiotocographic abnormalities	17 (2 unknown)	77 %	4 (7 unknown)	14 %
Perinatal use of corticosteroids	2	9 %	8	28 %
Unfortunate Apgar score (5 minutes)	1 (2 unknown)	5 %	1 (2 unknown)	3 %
Blood group incompatibility	0	0%	0	0 %
Infant respiratory distress syndrome	5	23 %	16	55 %
Sepsis	8	36 %	13	45 %
Convulsions	0	0%	0	0%

Table 3: Left and right renal size within the three study groups

	SGA Mean (SD)		AGA vs SGA Mean difference (95% CI)		AGA Mean (SD)		Controls vs AGA Mean difference (95% CI)		Controls Mean (SD)		Controls vs SGA Mean difference (95% CI)	
All subjects	N = 22				N = 29				N = 30			
Left renal size												
Absolute kidney length (cm)	10.0	(0.7)	0.4	(-0.2;0.9)	10.4	(1.1)	0.7	(0.1;1.2) †	11.1	(1.0)	1.0	(0.5;1.5) ‡
Relative kidney length (cm/m)	6.0	(0.4)	0.0	(-0.3;0.2)	6.0	(0.6)	0.3	(0.0;0.6) †	6.3	(0.6)	0.3	(0.0;0.6) †
Absolute kidney volume (ml)	123.8	(37.1)	5.3	(-12.4;23.0)	129.1	(25.8)	31.5	(13.1;49.8) ‡	160.5	(42.4)	36.8	(14.1;59.5) †
Relative kidney volume (ml/m^2)	73.5	(19.3)	-2.0	(-10.6;6.7)	71.5	(11.3)	14.7	(5.4;24.0) ‡	86.2	(22.4)	12.8	(0.8;24.7) †
Cortical thickness (cm)	0.9	(0.2)	0.0	(-0.2;0.1)	0.9	(0.3)	0.1	(-0.0;0.4)	1.1	(0.4)	0.1	(-0.1;0.3)
Right renal size												
Absolute kidney length (cm)	10.2	(0.9)	0.1	(-0.3;0.6)	10.3	(0.7)	0.3	(-0.2;0.7)	10.6	(1.0)	0.4	(-0.1;0.9)
Relative kidney length (cm/m)	6.1	(0.4)	-0.2	(-0.4;0.1)	5.9	(0.4)	0.1	(-0.1;0.3)	6.0	(0.5)	-0.1	(-0.3;0.2)
Absolute kidney volume (ml)	126.2	(43.5)	8.0	(-12.5;28.5)	134.1	(29.4)	15.6	(-4.8;36.1)	149.7	(46.8)	23.6	(-2.0;49.2) *
Relative kidney volume (ml/m^2)	74.6	(22.4)	-0.3	(-10.3;9.7)	74.2	(12.9)	5.6	(-3.9;15.1)	79.9	(22.2)	5.3	(-7.3;17.8)
Cortical thickness (cm)	0.8	(0.2)	0.0	(-0.2;0.1)	0.8	(0.3)	0.2	(0.0;0.4) *	1.0	(0.4)	0.2	(-0.0;0.4) *

Data expressed by mean ± standard deviation, mean difference and 95% CI
SGA: Small for gestational age; AGA: Appropriate for gestational age;
* P value < 0.10, † P-value < 0.05, ‡ P-value < 0.01

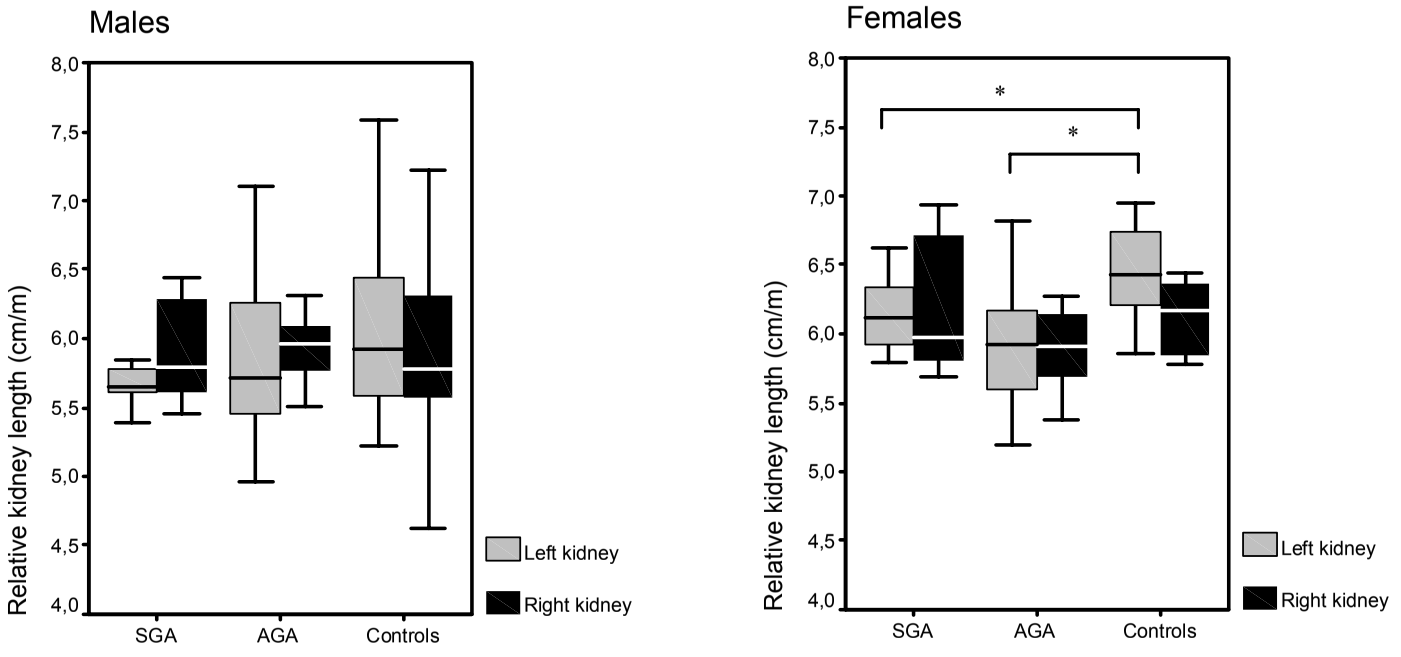

Figure 1: Relative Left and Right Kidney Length (cm/m) in males (Figure 1A) and females (Figure 1B).
In females left relative kidney length was smaller in SGA and AGA compared with controls, but not in males. * P-value < 0.05.

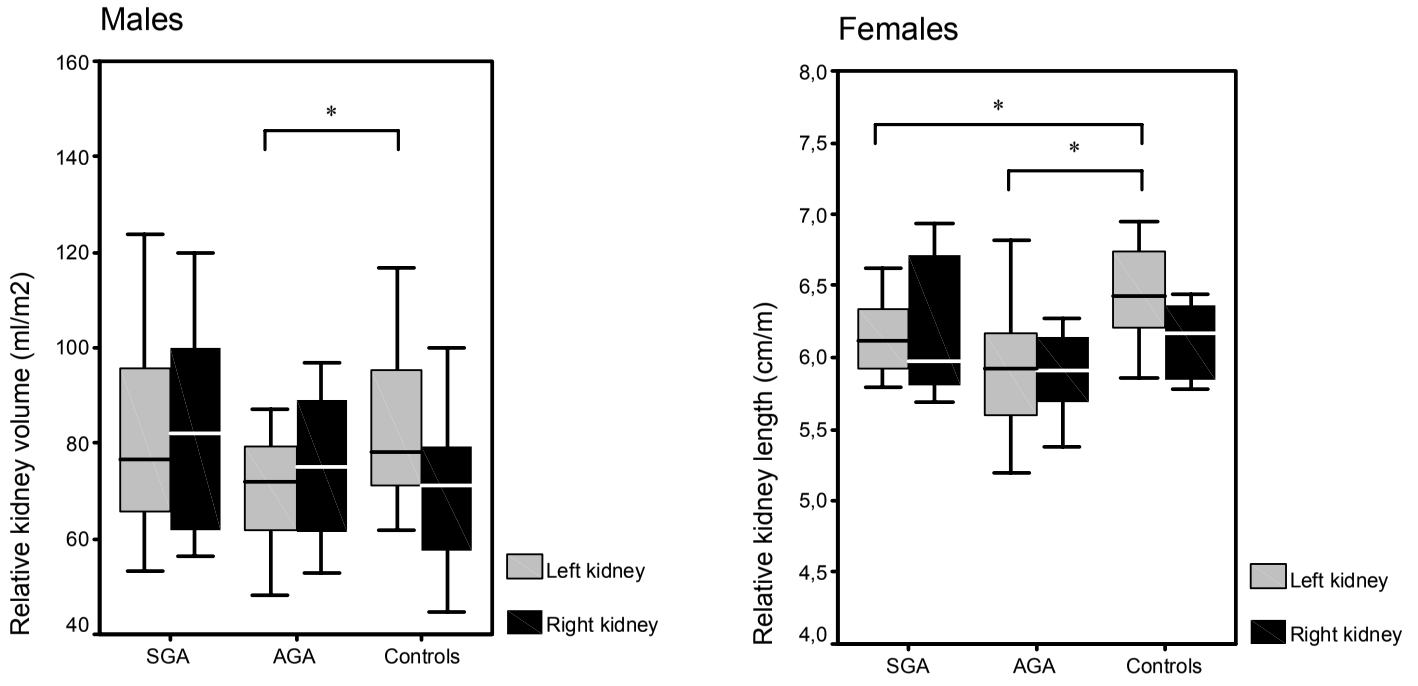

Figure 2: Relative Left and Right Kidney Volume (ml/m^2) in males (Figure 2A) and females (Figure 2B).
In males only left relative kidney volume differs between AGA and Controls. In females, both left and right relative kidney volume differs between SGA and controls and between AGA and controls. * P-value < 0.05.

contrast, in AGA and SGA preterms this percentage was 48% and 41% respectively. As shown in Table 3, SGA and AGA preterms had significant smaller absolute and relative left renal length and volume compared with controls. These observations were more obvious in females as shown in Figure 1 and 2. Absolute and relative right kidney volume was significantly smaller in SGA and AGA participants compared with controls, but especially in females. No significant difference between SGA and AGA subjects in left and right kidney size was present.

Renal and ureterovesical anomalies

Subclinical anomalies of the kidneys and ureters were present in 8 of 51 subjects born prematurely, and not in controls (Fisher exact p-value = 0.02). Anomalies varied from nephrocalcinosis (n=1), unilateral pyelocaliceal dilatation (n=3), ureteropelvic junction obstruction (n=1), ureter dilatation (n=1), extra-renal pelvis (n=1), ureter duplication and ureterocèle (n=1). None of the subjects were known with these anomalies before the renal ultrasound was performed. No difference in prevalence of renal abnormalities between SGA (4/22) and AGA (4/29) individuals was present.

DISCUSSION

We found that absolute and relative renal length and volume is decreased in 20-year-old individuals born very prematurely. The difference in left kidney size was larger than the difference in right kidney size, and was more obvious in females. No significant effect of IUGR on renal size was found within the individuals born very prematurely. These results indicate that fetal kidney development is impaired after preterm birth and the suggested effect of IUGR on renal size is probably small within these very preterm born individuals. This may be due to the fact that IUGR in these preterms was not present during nephrogenesis, which peaks at 32nd weeks of gestation and continues until 36 weeks of gestation and is correlated to renal size.[34]

Recently, Singh et al. described that lower renal volume in aboriginals represents kidneys with reduced nephron number [34]. It has been demonstrated that postnatal nephrogenesis in preterm born individuals is limited.[63;64] Therefore, a reduced nephron number after preterm birth probably persists throughout life. In 56 deceased extremely premature infants and 10 deceased full term born controls, nephron number was highly correlated to gestational age and active glomerulogenesis was ceased after 40 days postnatal.[63] The finding that decreased nephron number predisposes to reduced renal function at adult age underlines the importance of our results.[34;45] Therefore, preterm born individuals may have higher risk of renal

disease in later life. This finding concurs with our previous results of increased blood pressure and unfavourable renal function and microalbuminuria in these premature born individuals.[132;133]

Decreased renal volume was also found in low birth weight (LBW) aboriginal children between 5-18 years of age.[146] The BSA adjusted renal volume was 78.5 ml/m^2 in 33 LBW subjects, compared with 85.7 ml/m^2 in 141 normal birth weight (NBW) subjects (p value 0.018), but renal length was equal. It was stated that LBW in these subjects was a result of IUGR, but data on gestational age were unavailable in many participants. In contrast, we have found that IUGR preterm subjects did not have significant smaller renal size than non-IUGR preterm born subjects.

In our study, renal size differences between preterms and controls were more obvious in the left kidney than in right kidney. Similar results were described in the same study in aboriginal children of Spencer et al. The relative left BSA adjusted kidney volume was 83.6 ml in LBW versus 93.5 ml/m^2 in NBW subjects (p-value 0.015) and relative right kidney volume was 73.3 ml/m^2 in LBW versus 77.9 ml/m^2 in NBW subjects (p-value 0.225).[146]

Interestingly, smaller absolute left kidney volume (but not right kidney volume) was also present in adult subjects with chronic kidney failure and patients with hypertension compared with normotensive chronic kidney failure patients.[150] It was not studied whether renal size in these hypertensive patients was associated with birth weight and gestational age. In an animal model it was shown that the kidneys respond asymmetricly to adrenoceptor stimulation after intrauterine stress.[27] Whether this phenomenon is also present in humans, and if (fetal) kidney growth is affected by this is not known.

We hypothesize that early postnatal renal growth is limited in very preterm born individuals, especially in left kidneys. Recently, it was demonstrated that postnatal renal growth after preterm birth was limited until 18 months of age, but no difference in left and right kidney growth was mentioned.[148] To our knowledge, only one study described fetal kidney growth separately for left and right kidney.[151] In this longitudinal study 246 normal fetal kidneys at several ages of gestation were sonographicly measured. No significant difference was found between left and right kidney growth between 16 and 38 weeks of gestation. It might be that the left kidney grows more rapidly than the right kidney after the gestational age of 32 weeks in normal gestation, but larger studies are needed to confirm this finding. If this difference in growth is not present after very preterm birth, it might explain why left renal size in our very preterm cohort (<32 weeks) is more affected than the right side through an anatomical and embryological pathway.

In human studies it has not been reported previously that the differences in renal size between IUGR and non-IUGR individuals was more obvious in females, but

similar findings were reported in an experimental IUGR rat model.[121] Kidney weight was more reduced due to IUGR in female rats (1.99 g in IUGR vs. 0.78 g in controls) than in male rats (3.33 g in IUGR vs. 2.79 in controls). Moreover, plasma creatinine levels were increased in these female IUGR rats, but not in IUGR males. These data indicate that males and females respond differently to IUGR, with respect to renal development and renal function. The data suggests that females have either smaller kidneys at preterm birth compared with males (limited intra-uterine renal growth in females), or that females are less capable of renal catch-up growth after preterm birth. The pathophysiological mechanism for this gender difference remains unexplained and needs further research.

Renal anomalies were more frequently present in the very preterm born participants (8/51) compared with the controls (0/30). The anomalies consisted mainly of ectasia or dilatation of the ureters and pyelocaliceal system. None of the participants were known with their renal anomalies, and all subjects were asymptomatic. In preterms some renal diseases, such as acute tubular necrosis and nephrocalcinosis, are more frequently shown, but an increased incidence of the renal anomalies we found was not reported previously.[152;153] It is not known whether the anomalies of the pyelocaliceal system and ureters were already present at (preterm) birth, or that renal development was disturbed early postnatal or throughout childhood while renal ultrasound during follow-up was not obtained. One possible biological pathway may originate from the observations that in-utero disturbance of the renin-angiotensin system affects normal kidney development. [49;50] The strength of our study was that we were able to evaluate the effect of prematurity separately from the effect of IUGR within the premature born individuals. The main difference in renal size was found between very preterm (AGA) and at term born participants. The additional effect of IUGR in these very preterm born subjects was small and not significant for all renal sizes. Therefore, we suggest that very preterm birth predisposes to decreased renal size at adult age, due to limited renal growth after birth.

A limitation of the study was that three different radiologists obtained the data of renal size. To minimize the variability between the three radiologists renal size was measured according a standardized protocol and all radiologist measured subjects in all three groups. The mean error in measurement was comparable to other studies.[154;155] Moreover, the results of renal length in controls were similar as reported by Miletic et al.[156], indicating accurate measurements of renal size in our participants.

In conclusion, kidney development after preterm birth is probably limited, leading to (relatively) smaller kidneys and a higher frequency of renal structural anomalies at the age of 20 years. IUGR did not attenuate these observations in these preterm born individuals. The differences were more obvious in left kidneys, and in females. Detailed measurement of renal development, in both size and histological changes,

in a large epidemiological study design is required to underline the pathophysiological mechanism of unilateral decreased renal growth after very preterm birth.

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Chapter 5

General Discussion, Future Perspectives and Clinical Implications

GENERAL DISCUSSION

The aim of the work described in this thesis was to evaluate early determinants of blood pressure, renal function and renal size in young adult individuals who were born very premature. In this chapter the six research questions, as formulated in the general introduction, are answered. Future perspectives and clinical implications of the findings are discussed afterwards. The main findings of this thesis are summarized in the textbox below.

MAIN FINDINGS

- The mean systolic blood pressure and prevalence of hypertension is increased in individuals who were born very premature compared with full term born controls.
- IUGR does not affect blood pressure within individuals who were born very premature.
- Glomerular filtration rate is unfavorable influenced and microalbuminuria is increased in individuals who were born very premature and after IUGR, compared with individuals who were born premature with appropriate birth weight.
- Renal functional reserve capacity and effective renal plasma flow are decreased in individuals who were born very premature and IUGR compared with full term born controls, suggesting impaired nephron number with single nephron hyperfiltration due to persistent afferent vasodilatation.
- Renal size is decreased in individuals who were born very premature, compared with full term born controls. Left kidneys seem more affected than right kidneys, and women more than men.
- The number of renal anomalies in our study is higher in individuals who were born very premature compared with full term born controls.
- Renal size is associated with renal function in our study population.

Two studies were carried out between April 2002 and November 2004 in The Netherlands to answer the research questions. The POPS 19 study is a follow-up study at the age of 19 years of a national birth cohort consisting of individuals who were born very premature (GA < 32 wk) and/or with very low birth weight (< 1500 g). Estimated GFR, microalbuminuria and blood pressure values were obtained in 422 individuals (only GA < 32 wk) at the age of 19 years. The POPS Nephrology study contained 52 of these participants (23 SGA and 29 AGA) and 30 full term born con-

trols at the age of 20 years. Data on renal function (GFR and ERPF) before and after renal stimulation, microalbuminuria, renal ultrasound and 24-hour ambulant blood pressure monitoring were obtained.

Blood pressure in young adult individuals who were born very premature.

1. Is IUGR a predisposing factor for increased blood pressure at young adult age within individuals who were born very premature?
2. Is blood pressure increased at young adult age in individuals who were born very premature and IUGR compared with individuals who were born very premature with appropriate birth weight, and with full term born controls?

An inverse association between birth weight and cardiovascular risk and adult hypertension has been described frequently in the past.[11;12;14;90;91] In these studies individuals born at term with low birth weight had higher blood pressure values at adult age than subjects with higher birth weight. This effect was attenuated by accelerated (early) postnatal growth until the age of 1 year.[7] Underdevelopment of organs and organ systems (such as kidneys, pancreas and vascular system) in individuals born after IUGR may lead to permanent alterations in these organs and increase the susceptibility of adult disease.[23] A limited number of nephrons is demonstrated in subjects born after IUGR, which may increase systemic and intraglomerular blood pressure to maintain normal renal function, but renal damage and function loss may occur ('hyperfiltration' theory).[36;37;157]

As kidneys develop until the last phase in pregnancy[62], and postnatal nephron development in these very preterm born individuals is probably limited[63;64], it is likely that preterm birth alone is also accompanied with a decreased number of nephrons throughout life. The effect of gestational age and of IUGR within very premature born individuals on the development of adult hypertension was evaluated in only a few studies.[67;68]

The results from the POPS 19 and POPS Nephrology studies both have demonstrated that young adult systolic blood pressure was increased in individuals who were born very premature, but that IUGR did not affect blood pressure within these very preterm born individuals. *(Chapter 3.2 and 4.2)* In both studies the mean systolic blood pressure of the very preterm born individual was 123 mmHg, compared with 120 mmHg in full term born participants of the POPS Nephrology study. Also, the prevalence of hypertension was higher in the very premature born individuals (10% in the POPS 19 study, and 6% in the POPS Nephrology study). Within the very preterm born participants potential confounding variables, such as maternal hypertension during pregnancy and maternal smoking during pregnancy, did not affect adult blood pressure.*(Chapter 3.2)* Furthermore, no effect of (early) postnatal

catch-up growth was found within this very preterm born cohort. The best predicting factor for adult blood pressure in our cohort was current weight. *(Chapter 3.2)* This was evaluated by using a regression model with the 'unexplained residuals' for adult weight dependent on birth weight *(Chapter 2)*, to prevent improper adjustment for adult weight (causal pathway).

Nowadays, in individuals who were born very preterm or small for gestational age and develop short stature during childhood and adolescence, growth hormone therapy is frequently used to increase adult height. However, it is not known whether this increase in adult height, and thus weight, will influence the risk of high blood pressure. Within the participants of the POPS studies (all born in 1983), growth hormone therapy was not used for this purpose yet. New studies are indicated to elucidate the effect of growth hormone therapy in SGA premature individuals on adult blood pressure.

In summary, small differences in systolic blood pressure as a result of very premature birth, but not IUGR, are already detectable at young adult age, but current weight predicts adult blood pressure best.

Renal function in young adult individuals who were born very premature.

3. Is IUGR a predisposing factor for decreased renal function at young adult age within individuals who were born very premature?
4. Are renal function and renal functional reserve capacity (RFRC) impaired in individuals who were born very premature and IUGR compared with individuals who were born very premature with appropriate birth weight, and with full term born controls at young adult age?

Decreased renal function and nephron number after IUGR birth was demonstrated in several experimental and a few human studies.[45;46;113;114;157] As mentioned above, evidence is accumulating that IUGR and nephron endowment have an important role in the development of adult hypertension and renal disease in adult life. [34;36;37;42;43;157] Also in low birth weight children higher relapse rates of nephrotic syndrome have been reported.[158] In IgA nephropathy the percentage of sclerotic glomeruli is higher combined with an increased blood pressure in low birth weight children.[159] Studies evaluating the effect of prematurity on future renal function remain scarce and inconclusive.[66;143]

In the POPS 19 study it was demonstrated that small but significant differences in renal function (estimated GFR and microalbuminuria) were present between the individuals who were born very premature and small for gestational age (SGA) and the premature born participants with appropriate birth weight (AGA): Lower GFR

and higher microalbuminuria. *(Chapter 3.3)* These differences in (baseline) GFR were similar, but not significant, in the POPS Nephrology study.*(Chapter 4.3)* In the latter study the increase in GFR after renal stimulation tended to be lower in the SGA participants compared with controls. These data suggest that at the age of 20 years single nephron (SN) hyperfiltration is present at baseline, and thus a decreased RFRC, in the SGA premature born individual. The capacity of efferent renal vasodilatation due to dopamine infusion was intact in all groups, as the FF fell, and the increase in ERPF was equal. SN hyperfiltration may therefore better be explained by a decreased capacity of afferent vasodilatation, which may originate from a situation of persistent baseline afferent vasodilatation, resulting in a limited or absent effect of amino-acids. Persistent baseline afferent vasodilatation is accompanied with an increased intraglomerular pressure (increasing the risk of renal damage and renal function loss in later life)[41;160], which concurs with the finding that baseline FF was higher in the premature participants compared with controls. *(Chapter 4.3)* SN hyperfiltration is probably needed to maintain a normal GFR, and an indication that the number of nephrons is decreased, which also fit with the observations that baseline ERPF was lower in SGA preterms compared with full term born controls. In addition, microalbuminuria was more overt in SGA preterms compared with AGA preterms. *(Chapter 3.3 and 4.3)*

The decreased RFRC, ERPF with persistent afferent vasodilatation, increased microalbuminuria and systolic blood pressure at this young age, and the knowledge that renal function decreases during life, suggests that very premature SGA born individuals are at increased risk for progressive renal disease during life.[120;122] Future prospective studies of these individuals are warranted to evaluate their renal function over time. Additional studies on the effectiveness of screening programs for secondary prevention of progressive renal disease in this population may be of important value.

Renal size in young adult individuals who were born very premature

5. Do individuals who were born IUGR and / or prematurely have smaller (relative) kidney size at young adult age compared with full term born controls?

In Aboriginal communities a strong relation between birth weight and renal size, nephron number, albuminuria and systolic blood pressure has been shown.[34] Renal size was also reduced in low birth weight aboriginal children, suggesting an increased risk of renal diseases during life.[34;146]

To evaluate whether renal size at young adult age was impaired in individuals who were born very premature and IUGR we estimated renal size with a renal sonography in the participants of the POPS Nephrology study.*(Chapter 4.4)* It was demonstrated that both absolute and relative renal length and volume were smaller in

the individuals who were born very premature (either SGA or AGA) compared with controls. Surprisingly, these differences were more obvious in the left kidney and in women. Though some other studies have found a similar effect of IUGR and gender on kidney size, no underlying mechanism is known.[146;161] Two mechanisms are suggested to be involved. First, left and right fetal kidney growth may differ during the three trimesters of pregnancy and may therefore be affected asymmetricly in IUGR and preterm born individuals. However, no conclusive studies on fetal kidney growth are available. Additionally, postnatal kidney growth may also be impaired after IUGR and very preterm birth.[148] Second, the left and right kidney may respond asymmetricly to adrenoceptor stimulation after intrauterine stress, as shown in an animal model.[27] Whether this phenomenon is also present in humans, and whether (fetal) kidney growth is also affected by these stimuli is not known. Further studies are warranted.

Interestingly, the number of renal anomalies in the very preterm born individuals was higher compared with the controls. Renal anomalies mainly consisted of ectasia or dilatation of the ureters and the pyelocaliceal system, suggesting a disturbance in renal development. As it is not known whether these anomalies were already present at birth, we cannot determine whether this disturbance in renal development was induced prenatal or postnatal. Potential prenatal causes of impaired renal development may include maternal medication use, such as medications inhibiting the RAS, corticosteroids and gentamycin. Administration of these drugs and inadequate postnatal nutrition (such as deficiency of Vitamin A) in the postnatal period also could have influenced renal development.[28;49-52]. Future studies should focus on such determinants affecting prenatal and postnatal renal development (in size and structure) in premature and at term born individuals.

Associations

6. Is there an association between renal size, renal function, renal functional reserve capacity, and blood pressure in very preterm born individuals and in controls? or Do subjects with an increased blood pressure also have a decreased renal function and a decreased renal size?

In the studies described in this thesis it was demonstrated that increased blood pressure and a decreased renal size was present in both SGA and AGA preterm born individuals compared with controls *(Chapter 4.4)*. Renal function was decreased most in SGA preterm individuals *(Chapter 3.3 and 4.3)*. Renal size was significantly associated with renal function, but not with blood pressure.*(Chapter 4.3)*. Renal function was not related to blood pressure either.

These results indicate that rise in blood pressure and renal function loss does not develop simultaneously in these individuals: blood pressure increase precedes renal

function loss. These observations were also shown in some animal studies.[144;145] In uninephrectomized rats blood pressure increased before renal function decreased compared with control rats.[144] As blood pressure was high in AGA subjects, this would suggest that these individuals are also at increased risk for renal function loss. The most plausible explanation would be that nephron number at birth is impaired in very premature born individuals, but more in SGA than in AGA preterms. However, it is possible that the increase in blood pressure and decrease in renal function after IUGR birth do not origin from the same biological mechanism.[33]

Other parameters of the Metabolic syndrome within the POPS 19 study

Besides blood pressure and renal function, other parameters of the metabolic syndrome were also studied within the POPS 19 study. It was demonstrated that the insulin resistance at the age of 19 years in very preterm born individuals was predicted by rapid postnatal weight gain until 3 months post term and by adult body composition.[162] In contrast, no association between birth weight (SDS), early postnatal growth or gestational age and lipid profile or intima-media thickness of the carotid arteries at 19 years of age was found.[163]

In summary, the studies described in this thesis suggest that premature birth affects renal function and blood pressure at (young) adult age, and especially when born both SGA and premature. Minor differences are already detectable at young adult age. The biological mechanism is likely to originate from a decreased renal development, with alterations of vascular structure and limited nephron number.

FUTURE PERSPECTIVES

Other pathways than nephron endowment, such as programming of the hypothalamic-pituitary-adrenal axis and glucocorticoid receptor, dysfunction of the pancreatic β-cells, RAS, and endothelium, may also be involved in the development of hypertension and renal disease.[20;58;59;164;165] Additional analyses within the POPS 19 participants will be addressed in order to evaluate whether associations between blood pressure, GFR, microalbuminuria and other parameters of the metabolic syndrome (such as insulin sensitivity, dyslipedemia, fat distribution, and obesity, intima-media thickness), and genetic polymorphisms are present.[166-168]

Future follow-up of participants of the POPS studies may be very useful in the evaluation of progress in renal function loss over time. Furthermore, measurements of renin and angiotensin I and II concentration in newborns of all birth weights and ranges of gestation and prospective evaluation of their renal function and blood

pressure might improve insight in the influence of the RAS in the 'developmental origins of adult health and disease' hypothesis. [58;59] Also, insight in optimal nutrition in the very preterm born individual would give the opportunity to improve postnatal growth and (organ) development. Finally, to understand the differences in left and right renal size evaluating normal renal growth would be of great value.

CLINICAL IMPLICATIONS

Prevention should focus primary on optimal fetal and early postnatal nutrition and prevention of premature birth. Secondary prevention can be established by follow-up of very premature born and low birth weight individuals, to trace and treat early signs of renal damage and hypertension. Minimal-invasive methods such as measurement of microalbuminuria in triplet urine spots, measurement of blood pressure, and estimation of GFR by obtaining blood samples to measure serum creatinine levels should be considered. Microalbuminuria, hypertension and decreased GFR should be monitored. Treatment with angiotensin converting enzyme (ACE) inhibitors or Angiotensin II receptor blockade drugs may be needed in individuals with persistent microalbuminuria and hypertension. Treatment with ACE inhibitors has demonstrated a decrease in the amount and progression of microalbuminuria and may therefore also prevent renal function loss.[169] Finally, birth characteristics should be obtained in all medical interviews evaluating the individual risk profile for cardiovascular and end-stage renal disease.

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SAMENVATTING

Bij verschillende studies is gesuggereerd dat groeiachterstand van het kind tijdens de zwangerschap leidt tot een verhoogde kans op het ontwikkelen van ziekten op latere leeftijd, zoals bijvoorbeeld hoge bloeddruk, vetzucht, hart- en vaatziekten, suikerziekte, en nierziekten. De achterliggende gedachte is hierbij dat bepaalde organen of orgaansystemen (zoals alvleesklier, nieren en de bloedvaten) onvoldoende zijn ontwikkeld op het moment van de geboorte, en dat deze achterstand niet meer wordt ingehaald. Aanpassingen van deze organen, om te kunnen overleven op de korte termijn, zouden op relatief jonge leeftijd leiden tot minder goede functie en (versnelde) schade. Deze hypothese is ook wel bekend als de 'Barker' hypothese, of de 'Fetal origins of adult disease' hypothese, of de 'Developmental origins of adult health and disease' hypothesis.

Tot nu toe is weinig onderzoek verricht naar de effecten van vroeggeboorte, groeiachterstand en de combinatie daarvan op de nierfunctie en bloeddruk op de langere termijn.

In dit proefschrift zijn de bevindingen met betrekking tot vroeggeboorte, groeiachterstand, nierfunctie en bloeddruk beschreven naar aanleiding van een algemeen onderzoek en een vervolgonderzoek bij jong volwassenen.

Het algemene onderzoek is bekend als de POPS 19 studie: aan alle nog in leven zijnde 959 personen die in 1983 meer dan 8 weken te vroeg zijn geboren, en / of een extreem laag geboortegewicht hadden (< 1500 gram) is gevraagd om deel te nemen aan een landelijke studie waarin zou worden gekeken naar de algemene gezondheid (waaronder bloeddruk en nierfunctie) op de leeftijd van 19 jaar (POPS 19 studie: Hoofdstuk 3).

Uiteindelijk hebben 596 personen aan dit algemene onderzoek meegedaan. Van deze 596 personen waren 422 personen geboren met een zwangerschapsduur korter dan 32 weken (groep 1), en 274 personen met een zwangerschapsduur langer dan 32 weken, echter met een geboortegewicht van minder dan 1500 gram (groep 2). Een aantal personen uit groep 1 is gevraagd aan het vervolgonderzoek deel te nemen (52 personen). In dit vervolgonderzoek zijn met een preciezere methode dan gehanteerd bij het algemene onderzoek de bloeddruk en nierfunctie gemeten. Bij dit onderzoek zijn tevens 30 personen betrokken die tussen 1982 en1984 geboren zijn en die niet te vroeg geboren zijn. (POPS Nefrologie studie: Hoofdstuk 4)

Uit het algemene onderzoek en het vervolgonderzoek is gebleken dat de gemiddelde bloeddruk van de personen die te vroeg geboren zijn hoger is dan bij diegenen die niet te vroeg geboren zijn. Het geboortegewicht heeft geen invloed op de gemiddelde bloeddruk.

De nierfunctie is minder gunstig bij de te vroeg geboren personen, waarbij de nierfunctie nog minder gunstig is naar mate men een lager geboortegewicht heeft. Dit uitte zich ook door meer eiwituitscheiding in de urine.

Daarnaast zijn de nieren kleiner en vaker afwijkend bij de te vroeg geboren personen. Het is goed mogelijk dat dit te maken heeft met een minder goede ontwikkeling van de nieren voor of vlak na de geboorte.

De uitkomsten van de onderzoeken suggereren dat de nieren van te vroeg geborenen met een laag geboortegewicht minder reserve hebben, waarbij waarschijnlijk een hogere druk bestaat in de nieren, waardoor de kans op nierschade op relatief jonge leeftijd groter is. Dit zou kunnen verklaren waarom deze personen meer eiwit in de urine hebben.

Verminderde nierfunctie kan leiden tot schade aan de nieren, hetgeen kan leiden tot verdere vermindering van de nierfunctie. De verwachting bestaat daarom dat op latere leeftijd de verschillen in nierfunctie tussen de personen die wel en die niet te vroeg geboren zijn groter zullen worden.

Het is dan ook verstandig om bij alle personen die extreem vroeg geboren zijn vanaf jong volwassen leeftijd regelmatig onderzoek te doen naar de nierfunctie (door bijvoorbeeld bloed- en urineonderzoek) omdat vroegtijdig starten van een behandeling kan bijdragen aan een minder snelle voortgang van het verlies aan nierfunctie.

NAWOORD

Met veel plezier heb ik de afgelopen jaren gewerkt aan de onderzoeken zoals beschreven in dit proefschrift. Door mee te werken aan verschillende onderzoeksprojecten tijdens mijn studie en het eerste jaar na mijn afstuderen, en het enthousiasme dat daarbij op mij is overgedragen, heb ik deze weg bewandeld. Ik beschouw het dan ook als een eer dat dit werk uiteindelijk heeft geleid hebben tot een proefschrift ter verkrijging van de graad van doctor.

Bij de uitvoering van het POPS 19 onderzoek zijn meerdere instanties betrokken geweest. Hierbij is de inzet van vele personen met ieder zijn eigen expertise (logistieke coördinatie, werving van studiedeelnemers, verzamelen van gegevens, metrologie, epidemiologie en statistiek) van groot belang geweest om tot een mooi resultaat te kunnen komen. Dank aan eenieder die een bijdrage heeft geleverd.

Bij het 'deelproject nieren', de POPS Metrologie studie, zijn meerdere afdelingen en dus personen van het ErasmusMC-Sophia betrokken geweest om het onderzoek logistiek voor elkaar te krijgen. Ik denk daarbij onder andere aan de personen die tussendoor een echo inplanden en verrichtten, bierstulken bakten, cito mierfunctiesbepaalden, de bloeddrukmeters uitleenden en 'tijdig' bij de patiënten vele bloeden urinemonsters afnamen. Ik waardeer deze inzet enorm.

Ik heb de mogelijkheid gekregen om met mijn eigen ideeën onderzoek uit te voeren, ondertussen klinische vaardigheden op te doen in de kindergeneeskunde op de spoedeisende hulp en onderwijs en cursussen te volgen in de epidemiologie. De combinatie van leuk werk en ondertussen veel lol met vrienden, kamergenoten, andere collega's, en klinische en niet-klinische supervisoren, alias 'bazen', zijn voor mij de belangrijkste drijfveren geweest om tot een mooi einde van dit onderzoeksproject te kunnen komen. De steun en oneindig vertrouwen die ik hierbij van mijn thuisfront heb (gekregen) zijn voor mij zeer waardevol.

Als er iets te vieren valt, dan geef je een feestje.....

Muziek!

NAWOORD

Met veel plezier heb ik de afgelopen jaren gewerkt aan de onderzoeken zoals beschreven in dit proefschrift. Door mee te werken aan verschillende onderzoeksprojecten tijdens mijn studie en het eerste jaar na mijn afstuderen, en het enthousiasme dat daarbij op mij is overgedragen, heb ik deze weg bewandeld. Ik beschouw het dan ook als een eer dat dit werk uiteindelijk heeft geleid hebben tot een proefschrift ter verkrijging van de graad van doctor.

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Bij het 'deelproject nieren', de POPS Nefrologie studie, zijn meerdere afdelingen en dus personen van het ErasmusMC-Sophia betrokken geweest om het onderzoek logistiek voor elkaar te krijgen. Ik denk daarbij onder andere aan de personen die tussendoor een echo inplanden en verrichtten, biefstukken bakten, cito nierfuncties bepaalden, de bloeddrukmeters uitleenden en 'tijdig' bij de patiënten vele bloed- en urinemonsters afnamen. Ik waardeer deze inzet enorm.

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Als er iets te vieren valt, dan geef je een feestje…..

Muziek!

CURRICULUM VITAE

Mandy Gabriëlle Veen is geboren op 25 augustus 1975 te Delft. Zij heeft haar VWO diploma gehaald aan het Sint-Stanislascollege te Delft in 1993. Na het behalen van haar propedeuse Biomedische Wetenschappen aan de Universiteit Leiden in 1994 is zij gestart met de opleiding Geneeskunde aan dezelfde universiteit. Gedurende haar opleiding is zij betrokken geweest bij diverse onderzoeksprojecten. (LUMC, afdeling Neonatologie (dr. AJ de Beaufort) en Kindergastro-enterologie (dr. JJ Schweizer) en Spaarne Ziekenhuis Haarlem (Algemene kindergeneeskunde, dr. R Veenhoven)).

Na het afronden van haar studie Geneeskunde in 2001 is zij gaan werken als arts-onderzoeker op de afdeling Infectieziekten en Immunologie van het ErasmusMC-Sophia te Rotterdam (Prof.dr. R de Groot) en Vaxinostics BV (dr. HC Rümke) waar zij heeft meegewerkt aan vaccinatiestudies bij kinderen. In januari 2002 is zij gestart met haar promotieonderzoek, zoals beschreven in dit proefschrift, bij de afdeling Kindernefrologie van het ErasmusMC-Sophia (Prof.dr. AJ van der Heijden) in samenwerking met de afdeling Klinische Epidemiologie van het LUMC (dr. FW Dekker). Tijdens dit promotieonderzoek heeft zij de opleiding tot epidemioloog gevolgd (Prof.dr. JP Vandenbroucke), en werkte zij als arts op de spoedeisende hulp van het ErasmusMC-Sophia. Sinds september 2005 is zij werkzaam als arts-assistent op de afdeling kindergeneeskunde van het ErasmusMC-Sophia, alwaar zij haar opleiding tot kinderarts is gestart per 1 januari 2006 (dr. M de Hoog).

Mandy is getrouwd met Martin Keijzer. Zij hebben een dochter, Femke.

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