Chapter 1

General introduction
INTRODUCTION

Preterm birth interrupts the normal fetal growth and developmental processes of many organs and regulating systems. In early postnatal life, preterm born infants have impaired growth in comparison to the normal fetal growth in utero during the same period. Barker et al. (1) first indicated that impaired fetal growth is associated with an increased prevalence of the metabolic syndrome in later life. This is caused by fetal adaptations to the limited supply of nutrients that lead to changes in structure and metabolism (programming). These changes are functional for survival in the adverse intra-uterine environment, but may be permanent and lead to diseases in later life, including coronary heart disease, type 2 diabetes and hypertension. This so called Barker hypothesis, is nowadays called Developmental Origins of Health and Disease (DOHaD) hypothesis (https://dohadsoc.org/). In preterm born children, the impaired growth that takes place in the postnatal period corresponding with the last trimester of pregnancy might therefore result in comparable adaptations as in third trimester intra-uterine growth restriction.

The combination of preterm birth itself, postnatal morbidity and stress, and impaired postnatal growth may result in changes in structure and function of organs and regulating systems and these changes eventually could have consequences for later health. Studies in preterm born infants in later life indeed have shown a higher prevalence of components of the metabolic syndrome compared to controls (2-8). Moreover, the risk of long-term consequences of preterm birth is likely to increase with shorter gestational age. Johansson et al. (4) indeed showed that the risk of high blood pressure in young men increased with decreasing gestational age.

It is unknown whether the long-term consequences of preterm birth and programming in the postnatal period are already detectable in early childhood. We therefore decided to focus our investigations on the endocrine and metabolic consequences of preterm birth in infancy and early childhood in infants born with very-low-birth-weight (VLBW) (birth weight < 1500 g).

Hypothalamic-pituitary-gonadal axis

The fetal development of the hypothalamic-pituitary-gonadal (HPG) axis has been reviewed by Forest et al. (9). In brief, in male fetuses the testicles differentiate near the 7th week and the production of testosterone begins towards the 8th week of gestation. Increasing testicular activity corresponds to the first trimester peak of human chorionic gonadotropin (hCG) secretion. This period of active testicular testosterone secretion coincides with genital differentiation. Testosterone production reaches a maximum at 11-16 weeks and declines thereafter during midpregnancy. In female fetuses, differentiation of the ovary starts later than the testis and oocytes appear from about the 12th
week. Steroidogenesis in the fetal ovary is minimal. Testosterone levels at midgestation are lower in female than in male fetuses and levels of plasma oestrogens in female fetuses do not differ significantly from those in male fetuses (9).

Gonadotropins are detected in the fetal pituitary from the 10th week of gestation and released into the fetal circulation from the 11th-12th week of gestation. In midgestation, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels are significantly higher in female than in male fetuses (9, 10). During the last part of gestation, LH and FSH levels decrease to prepubertal values in both male and female fetuses (10). Free circulating concentrations of testosterone increase in both female and male fetuses with disappearance of the difference between sexes. Increased adrenal or placental secretion and change in clearance, receptor binding and binding to transport proteins may contribute to this rise of free testosterone levels (10). Testosterone and gonadotropin levels are inversely correlated in both sexes, indicating that testosterone plays an important role in controlling LH and FSH secretion by negative feedback mechanism (10).

After birth, the HPG axis is temporarily activated during the first months of life. This postnatal activation of the HPG axis is considered as an important phase in the maturation of the gonads and may play a significant role in the development of reproductive function. Several studies describe this postnatal activation in term born infants (11-17). In boys, this activation has been shown to be associated to the development of the testes as well as the penis and the scrotum (18-22) and as a consequence, the postnatal activation may be of importance for reproductive function in adult men. In girls, the exact importance of this postnatal activation for the development and function of the ovaries and for future fertility is still unclear.

In preterm born infants, the postnatal activation of the HPG axis seems to be exaggerated compared to full-term born infants (20, 23-26). The effect of prematurity on this postnatal activation is probably more distinct as the gestational age decreases, but data about the postnatal activity of the HPG axis in preterm infants born at a gestational age less than 30 weeks are limited. The consequences of the exaggerated activation of the HPG axis in preterm boys and girls for later function of the HPG axis, puberty and reproductive function are still unclear.

**Metabolic syndrome**

Over the last decades, there is growing awareness for the metabolic syndrome and the prevalence of the metabolic syndrome is increasing in parallel with changing life style and rising incidence of obesity. The metabolic syndrome is a combination of abnormalities in metabolic parameters, body size and blood pressure and is associated with an increased risk of type 2 diabetes and cardiovascular disease (27). Besides life style and obesity, fetal and early postnatal growth are determinants of the metabolic syndrome. Both term small-for-gestational-age (SGA) infants and preterm born infants have an
increased prevalence of several components of the metabolic syndrome in adulthood (2-4, 28, 29).

Insulin resistance plays a central role in the metabolic syndrome and adults born SGA or preterm are more insulin-resistant than controls (2-4, 28-30). In term SGA born infants, insulin sensitivity is already reduced in childhood, especially in children with catch-up growth, and also some of the other metabolic syndrome components are already present in childhood (31-37).

Reduced insulin sensitivity has also been demonstrated in preterm born children between the ages of 4 and 10 years (5). Of the other metabolic syndrome components, only blood pressure has been studied in preterm born children and was found to be higher compared to term born controls or compared to published reference ranges, even in early childhood (6, 8, 38, 39). So far, no studies have been published indicating that the other components of the metabolic syndrome, including reduced insulin sensitivity, in later life of preterm born infants can already be detected in early childhood.

**Hypothalamic-pituitary-adrenal axis**

The fetal development of the hypothalamic-pituitary-adrenal (HPA) axis has been reviewed by Bolt et al. (40). He described that the HPA axis, just like the HPG axis, becomes active early in gestation, when synthesis of steroids in the fetal adrenal cortex starts. During early gestation, levels of 3ß-hydroxysteroid dehydrogenase in the fetal adrenal cortex are low, resulting in relatively high levels of sulfated dehydroepiandrosterone (DHEAS). Expression of 11ß-hydroxysteroid dehydrogenase type 2 (11ß-HSD2), that converts active cortisol into inactive cortisone, is tissue-specific. The fetal adrenal cortex contains high levels, as well as the placenta, converting maternal cortisol into inactive cortisone, but tissues that need glucocorticoids for development (lung, adrenal medulla) have low levels of 11ß-HSD2. Fetal adrenal steroidogenesis is regulated by corticotrophin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH), which are produced by the fetal hypothalamus and pituitary, respectively and also by the placenta (40).

Programming of the HPA axis is one of the proposed mechanisms underlying the association between intra-uterine growth restriction and metabolic and cardiovascular consequences in later life. The inverse relation between birth weight and blood pressure, and consequently the importance of fetal growth for later blood pressure, was first indicated by Barker et al. (41, 42) and confirmed in many studies in children and adults, reviewed by Huxley et al. (43). Moreover, in adults birth weight is inversely associated with cortisol levels and cortisol levels are positively correlated to blood pressure (44, 45). These associations were also shown in children between the ages of 4.9 and 15.5 years and born at a gestational age > 32 weeks (46). Proposed mechanisms for the positive relation between increased HPA axis activity and blood pressure are activation of the central
sympathetic nervous system and reduced insulin sensitivity with hyperinsulinemia (47). The latter could be mediated by the stimulating effect of insulin on sympathetic nervous system activity, renal sodium retention and/or vascular smooth muscle growth (48).

The higher blood pressure in later life associated with preterm birth (2-4, 6-8, 38, 39) could also be caused by programming of the HPA axis. The association between cortisol and blood pressure in preterm born infants has been demonstrated in young adult men (49), but has not been investigated in preterm born infants < 32 weeks in (early) childhood.

In SGA born children, changes in the activity of 11ß-HSD2 probably contribute to the metabolic and cardiovascular consequences in later life. In SGA born children without catch-up growth, the cortisol/cortisone ratio at the mean age of 7 years was significantly higher compared to controls, suggesting a partial 11ß-HSD2 deficit (50). In these children, the cortisol/cortisone ratio was positively correlated with cholesterol levels, indicating a risk factor for cardiovascular disease (50). In preterm born children, the cortisol/cortisone ratio has not been investigated.

**Insulin-like growth factor I**

In early pregnancy, insulin-like growth factor II is the main factor for fetal growth and seems to be independent of nutrient supply (51, 52). Insulin-like growth factor I (IGF-I) is already detectable in many fetal tissues from the first trimester (53). However, IGF-I becomes the main determinant of fetal growth during the second half of pregnancy, which is demonstrated by a significant rise of IGF-I levels in the third trimester (54). In the fetus, hepatic IGF-I production is not regulated by growth hormone (GH), but insulin is the primary regulator. During the second half of pregnancy, the nutrient supply from the mother is very important for fetal growth, and stimulates IGF-I production directly and indirectly (through insulin) (51, 52). After birth, IGF-I remains important for growth and development. Regulation of IGF-I and growth becomes GH dependent, but in the first postnatal months growth is still primarily regulated by the glucose-insulin-IGF-I axis (52).

In contrast to term infants, the IGF-I levels in preterm infants decrease after birth and only increase gradually during the early postnatal period (55). Studies in preterm infants show that the IGF-I levels during the first postnatal weeks are positively related to early postnatal growth (56-60). Consequently, the low IGF-I levels in preterm born infants seem to play an important role in early postnatal growth restriction. This was confirmed by the study of Hansen-Pupp et al. (59) in preterm infants born at < 31 weeks gestation (mean gestational age 25.7 weeks). They showed that IGF-I concentrations were low during the first postnatal weeks and that this period corresponds to the phase of postnatal growth restriction. IGF-I levels increased at 30 weeks postmenstrual age, coinciding with initiation of catch-up growth for weight, length and head circumference. Nutrient intake
was not associated to growth and IGF-I levels during the initial phase of growth restriction, but was only correlated to growth and IGF-I levels during the phase of catch-up growth (59).

The majority of studies concerned with IGF-I levels in VLBW infants have focused on the early postnatal period. However, studies in mid-childhood (between 5 and 10 years of age) also suggest that lower IGF-I levels are related to prolonged growth restriction in VLBW infants (61, 62). Longitudinal data of IGF-I levels from the early postnatal period until early childhood in VLBW infants are limited.

**Challenges in data collection in young children**

Serial blood sampling in infants has major disadvantages, including the pain caused by the puncture and the risk of iatrogenic anemia, especially in VLBW infants, and is qualified as a burden. These factors limit the number of serial blood samples that can be drawn for research purposes from one infant in a period of time. However, to obtain accurate information about the pattern of hormone secretion in individuals, it is often necessary to collect serial samples. Samples that can be collected non-invasively and provide reliable information would be preferable. Both urine samples and saliva samples meet these criteria and might be used as alternatives for blood samples.

In conclusion, it is unknown whether the long-term endocrine and metabolic consequences of preterm birth are already detectable in early childhood. There is limited information available about the function of the neuroendocrine axes and the presence of the components of the metabolic syndrome in preterm born infants from birth to early childhood.

**AIMS OF THIS THESIS**

The primary aim of this thesis is to evaluate the endocrine and metabolic consequences of preterm birth in infancy and early childhood in VLBW infants. The hypothalamic-pituitary-gonadal axis, the hypothalamic-pituitary-adrenal axis, the growth hormone/insulin-like growth factor I axis and the components of the metabolic syndrome were studied. As the VLBW infants included in our studies were part of the Neonatal Insulin Replacement Therapy in Europe (NIRTURE) trial (63), we could also evaluate the effects of early insulin therapy on the neuroendocrine axes and on the components of the metabolic syndrome. Metabolic syndrome components and levels of cortisol, cortisone and IGF-I observed in the VLBW children, were compared to these parameters in term appropriate-for-gestational-age (AGA) born children. Finally, the reliability of salivary cortisol measurement as a non-invasive alternative to measurement in blood samples.
was investigated in the VLBW population. For evaluation of the HPG axis we only used a non-invasive method, as it has already been demonstrated that the measurement of gonadotropins in urine samples gives reliable results.

**STUDY DESIGN**

The VLBW infants were part of the Neonatal Insulin Replacement Therapy in Europe (NIRTURE) trial, an international multicenter randomized controlled trial investigating the role of early insulin therapy in VLBW infants (63). After written informed consent was obtained from both parents, VLBW infants younger than 24 hours of age and requiring intensive care were randomized to receive continuous intravenous infusion of insulin for the first 7 days of life or standard neonatal care with insulin treatment only in case of hyperglycemia. Exclusion criteria were maternal diabetes and major congenital anomalies. All infants participating in the NIRTURE trial in our neonatal intensive care unit were eligible for our studies. After discharge, all patients were followed in the outpatient clinic of the VU University Medical Center, with visits at the expected date of delivery and at the corrected ages of 3 months, 6 months, 1 year and 2 years. Approval from the ethics committee of the VU University Medical Center was obtained. The results of the NIRTURE trial did not show short-term clinical benefits of early insulin therapy (63); long-term results have not yet been published.

Anthropometry according to Dauncey et al. (64) was performed by a trained research nurse at all visits to the outpatient clinic. Blood pressure was measured at 2 years corrected age. Urine samples for measurement of FSH, LH, estradiol (girls) and testosterone (boys) were collected at the postnatal age of 1 week and 4 weeks, at the postmenstrual age of 32 weeks and at the corrected age of 0 months (expected date of delivery), 3 months and 6 months. Blood samples were taken at 6 months corrected age (for measurement of cortisol, cortisone, IGF-I and insulin) and at 2 years corrected age (for measurement of cortisol, cortisone, IGF-I, insulin, glucose, total cholesterol, HDL cholesterol and triglycerides). Salivary samples for measurement of cortisol were taken at 6 months and 2 years corrected age.

For the comparison of follow-up data of the VLBW infants, collected anthropometric, endocrine and metabolic data obtained from term born infants, included in another observational cohort study conducted by our department, will be used. The term infants of this reference population were born from a low-risk population of pregnant women included in a prospective longitudinal study (Trophoblast study) and recruited during the first trimester. The Trophoblast study aimed to investigate the use of circulating trophoblast for prenatal diagnosis of pregnancy-associated diseases such as preeclampsia (65). The term born infants were divided in AGA and SGA; SGA was defined as a birth
weight below the 10th percentile (66). In these term infants anthropometry was performed and blood samples were taken at 3 months, 1 and 2 years of age, according to the protocol of the Trophoblast study. IGF-I, cortisol and cortisone were measured in all blood samples; insulin, glucose, total cholesterol, HDL cholesterol and triglycerides were measured in blood samples taken at 1 and 2 years of age.

THESIS OUTLINE

Chapter 2 and 3 describe the postnatal activation of the hypothalamic-pituitary-gonadal axis in male (chapter 2) and female (chapter 3) VLBW infants by serial measurement of gonadotropins and testosterone/estradiol levels in urine samples from birth to 9 months of age and the effect of early insulin therapy on the postnatal activation of the hypothalamic-pituitary-gonadal axis.

Chapter 4 describes the prevalence of the components of the metabolic syndrome in VLBW infants at the corrected age of 2 years and the effect of early insulin therapy on the components of the metabolic syndrome.

In chapter 5, the components of the metabolic syndrome in VLBW infants at the corrected age of 2 years are compared to those in 2-year-old term AGA born children and the components of the metabolic syndrome in term SGA infants are compared to those in term AGA infants at 1 and 2 years of age.

Chapter 6 reports on the cortisol levels in VLBW infants at 6 months and 2 years corrected age, the correlation of cortisol levels to blood pressure at 2 years corrected age and the effect of early insulin therapy on cortisol levels. This chapter also investigates the reliability of salivary cortisol measurements in this population.

Chapter 7 compares the serum cortisol and cortisone levels and the cortisol/cortisone ratio in infancy and early childhood in VLBW infants to term AGA born infants and the relation of the cortisol/cortisone ratio to several metabolic syndrome components. In VLBW infants, the effects of early insulin therapy on the cortisone levels and the cortisol/cortisone ratio at 6 months and 2 years corrected age are also investigated.

Chapter 8 compares the IGF-I levels and its relation to growth parameters in infancy and early childhood in VLBW infants to term AGA born infants. In VLBW infants, the effects of early insulin therapy on the IGF-I and insulin levels at 6 months and 2 years corrected age are also investigated.

Chapter 9 discusses the conclusions of this thesis and the implications for future research.
REFERENCES


General introduction


