Chapter 9

General discussion
GENERAL DISCUSSION

Preterm birth has consequences for later health. The preterm birth itself, postnatal morbidity and stress, and the impaired postnatal growth may result in changes in structure and function of organs and regulating systems (programming). These changes are comparable to the changes that are caused by impaired fetal growth and may be the origins of diseases in later life, including coronary heart disease, type 2 diabetes and hypertension (1).

The studies in this thesis were aimed to evaluate endocrine and metabolic consequences of preterm birth in early life. The hypothalamic-pituitary-gonadal (HPG) axis, hypothalamic-pituitary-adrenal (HPA) axis, the growth hormone/insulin-like growth factor I (GH/IGF-I) axis and the components of the metabolic syndrome were studied in infancy and early childhood in children born with very-low-birth-weight (VLBW) (birth weight < 1500 g). As the VLBW infants were part of the Neonatal Insulin Replacement Therapy in Europe (NIRTURE) trial (2), the effects of early insulin therapy on the neuroendocrine axes and on the components of the metabolic syndrome were also investigated.

Hypothalamic-pituitary-gonadal axis

The postnatal activation of the HPG axis is exaggerated in preterm born infants compared to term born infants. Preterm born boys have higher levels of gonadotropins and testosterone during the first postnatal months in comparison with term born boys (3-5). This is associated with faster testicular and penile growth in early infancy in preterm boys (4). In preterm born girls, gonadotropin levels are higher during the first 10 weeks of life compared to term born girls, with follicle-stimulating hormone (FSH) levels 10-20 times higher and luteinizing hormone (LH) levels 3-4 times higher (5). In early infancy, preterm born girls also have higher estradiol levels than term born girls (6).

These differences in postnatal activation of the HPG axis between preterm and term born infants are probably caused by the more immature state of the HPG axis in preterm infants, which could be less sensitive for negative feedback by sex steroids and needs more time for full maturation of the inhibitory feedback system (3, 5). In preterm infant girls, ovarian folliculogenesis is delayed compared to term infant girls, therefore insufficient inhibitory feedback by ovarian inhibins and estrogens could also contribute to the higher and more prolonged FSH peak in preterm girls (7).

In contrast to the above mentioned studies conducted in populations of preterm infants with a wide range of gestational ages and birth weights, we studied the postnatal activation of the HPG axis in a group of only VLBW infants, all born at a gestational age of less than 30 weeks (chapter 2 and chapter 3). By using urine samples, we were able to collect serial measurements of gonadotropins and estradiol/testosterone levels without the burden of frequent blood sampling.
In male VLBW infants (chapter 2), levels of LH and FSH showed a peak at a mean postnatal age of 1 to 4 weeks (mean postmenstrual age of 30 to 32 weeks). Testosterone levels decreased with increasing age and, in contrast to earlier studies (3-5), did not show a significant peak. This could be specific for our population of male VLBW infants. The decrease of testosterone levels was faster in infants receiving early insulin therapy compared to those receiving standard care. This could be caused by the effect of insulin on sex hormone-binding globulin, or by a greater amount of adipose tissue in the early-insulin group, resulting in higher leptin levels and/or increased aromatization (8-11).

In female VLBW infants (chapter 3), both FSH and LH showed a peak at a mean postmenstrual age of 32 weeks, corresponding to a mean postnatal age of 4 weeks. Estradiol levels were highest in the youngest age group (mean postmenstrual age of 28 weeks) and decreased with increasing age. Peak gonadotropin levels were preceded by the decrease in estradiol levels resulting from the disappearance of placental estrogens, supporting the hypothesis that the rise in gonadotropin concentrations is caused by a decrease of inhibitory feedback by estradiol.

In conclusion, serial measurements of gonadotropins and estradiol/testosterone levels by making use of urine samples, provide an accurate description of the postnatal activation of the HPG axis in VLBW infants, without the burden of frequent blood sampling. Levels of LH and FSH show a peak in the first postnatal weeks in both VLBW boys and girls. These peak levels of gonadotropins in VLBW infants were measured at a comparable postnatal age as in previous studies in term born infants (12-14). Postnatal activation of the HPG axis does not depend on the postmenstrual age, indicating that birth itself, and not the degree of maturation, plays a crucial role in this activation.

**Metabolic syndrome**

Preterm born infants are at risk of the metabolic syndrome in later life. Several studies showed an increased prevalence of metabolic syndrome components, including reduced insulin sensitivity, in preterm born adults (15-23). As more information became available about the risks in adulthood after preterm birth, studies were extended to childhood. Reduced insulin sensitivity can already be detected in preterm born children between 4 and 10 years of age (24). This suggests that preterm born children of that age are already at risk of metabolic syndrome components, as insulin resistance plays a central role in the metabolic syndrome. Indeed, preterm born school children have higher blood pressure compared to term born controls (25) and to published reference ranges (26).

Younger preterm born children at preschool age might also be at risk of reduced insulin sensitivity and other metabolic syndrome components. Blood pressure is the only component that was earlier investigated at preschool age, and was already elevated in
early childhood in VLBW infants (27, 28). We evaluated the components of the metabolic syndrome in VLBW infants at the corrected age of 2 years (chapter 4 and chapter 5).

Chapter 4 describes the prevalence of the components of the metabolic syndrome, using reference values published earlier (29-32). The majority of the VLBW infants already had one or more components of the metabolic syndrome at the corrected age of 2 years. Especially the prevalence of raised blood pressure was high: 63% had systolic and/or diastolic blood pressure ≥ 90th percentile for age, sex and height. Approximately one third of the VLBW children had high triglycerides (≥ 0.98 mmol/l) and one third had low HDL cholesterol (≤ 1.03 mmol/l). Our data suggest that the high prevalence of metabolic syndrome components was not related to the prevalence of obesity, as none of the children had high BMI (BMI SDS > 2 for age and sex). Especially abdominal fat accumulation is associated with metabolic syndrome components (32), but measurements of waist circumference were not available in the population studied. Raised blood pressure and high triglycerides in VLBW children, using reference values published earlier (29-32), could result from reduced insulin sensitivity.

Chapter 5 describes the components of the metabolic syndrome at 2 years corrected age in VLBW infants participating in the NIRTURE trial (2), compared to those in 2-year-old term appropriate-for-gestational-age (AGA) born children obtained from the Trophoblast study (33), also conducted in our department. At 2 years corrected age, VLBW children had higher glucose levels than term AGA children. In both groups, some of the children had very high insulin levels, suggesting these were non-fasting values. Based on reference values of fasting insulin in children (34), we excluded the data from the children with high insulin levels. After exclusion of these data, VLBW children still had significantly higher glucose levels compared to term AGA children (data not shown). This suggests that VLBW children already have reduced insulin sensitivity at 2 years corrected age.

In this study, we could not confirm the results of our first study (high prevalence of high triglycerides and low HDL cholesterol). The cut-off values based on term born AGA children from our own population, turned out to be higher for triglycerides, lower for HDL cholesterol and lower for glucose, than the cut-off values used in our earlier study. This explains the difference in results of the two studies. The results of our second study are in our opinion more reliable, as in this study the cut-off values for lipids and glucose were based on a control group of term born children of the same age and from our own population.

At 2 years corrected age, VLBW children treated with insulin in the first week of life had lower triglycerides than children who received standard care and, for the subgroup with BMI SDS < 0 at 2 years corrected age, they also had higher HDL cholesterol levels. These findings suggest that early insulin treatment may have a more protective effect on the development of some of the risk factors of the metabolic syndrome at the age of 2 years.
In conclusion, in VLBW infants the higher prevalence of some of the components of the metabolic syndrome can already be detected at 2 years corrected age. They have a high prevalence of raised blood pressure, compared to earlier published reference values. VLBW children have significantly higher glucose levels than 2-year-old term born AGA children. Early insulin treatment may possibly have long-term benefits for some components of the metabolic syndrome in later life in VLBW infants.

**Hypothalamic-pituitary-adrenal axis**

Programming of the HPA axis possibly underlies the association between preterm birth and raised blood pressure in later life. In preterm born young adult men, the association between cortisol and blood pressure has been demonstrated (35). In children, the association between cortisol levels and blood pressure has only been shown in children between the ages of 4.9 and 15.5 years and born at a gestational age > 32 weeks (36).

We have shown that the association between cortisol and blood pressure in VLBW infants was already present at 2 years corrected age (Chapter 6). This supports the hypothesis that programming of the HPA axis may contribute to the high prevalence of raised blood pressure in early childhood in VLBW infants (Chapter 4) (27, 28). In our study, the prevalence of raised diastolic blood pressure was significantly higher in VLBW boys than in girls and the correlation between cortisol and blood pressure was only significant in boys, and not in girls, as was shown before in preterm born young adults (35). This sex difference is probably associated with the presence of sex hormones and the differential effects of estrogens and androgens on the renin-angiotensin system (37), as the HPG axis is also active in the postnatal period and in girls stays active during the first years of life (12, 38).

During the first 2 years of life, cortisol/cortisone ratio was significantly higher in VLBW children compared to term AGA children (Chapter 7). Higher cortisol/cortisone ratio suggests partial 11ß-hydroxysteroid dehydrogenase type 2 (11ß-HSD2) deficit and is related to several metabolic syndrome components, as shown earlier in SGA born children (39, 40). Therefore, low 11ß-HSD2 activity could also contribute to the long-term metabolic and cardiovascular risks of VLBW infants. The negative effect of cortisol on insulin sensitivity probably plays a crucial role in the association between high cortisol/cortisone ratio and metabolic and cardiovascular consequences (41, 42).

We found indications of long-term effects of early insulin treatment on cortisol and cortisone levels, possibly by affecting the programming of the HPA axis. Animal studies indeed have shown that increased insulin concentrations within the immature hypothalamus may lead to irreversible malprogramming (with morphological changes) of regulation centers for metabolism and body weight (43).

Our study confirms that measuring free cortisol in saliva is reliable and mirrors total cortisol in serum. Consequently, saliva can be used as non-invasive method for cortisol
measurements, allowing the collection of (serial) samples without the disadvantages of blood sampling.

In conclusion, in VLBW boys the positive correlation between cortisol and blood pressure is already present at 2 years corrected age, suggesting that programming of the HPA axis could contribute to the high prevalence of raised blood pressure in VLBW infants in early childhood. Early insulin treatment may affect this programming, resulting in higher cortisol and cortisone levels. In addition, VLBW infants have higher cortisol/cortisone ratio during early childhood compared to term born children, suggesting lower 11ß-HSD2 activity. This could contribute to the long-term metabolic and cardiovascular risks. Salivary cortisol mirrors serum levels at 6 months corrected age and has an important advantage as non-invasive method, especially in children.

**Insulin-like growth factor I**

In VLBW infants, IGF-I is not only involved in postnatal growth restriction, but also in growth restriction in mid-childhood (44, 45). IGF-I levels in mid-childhood in preterm born infants compared to term born infants were found lower in one study (45), but higher in another study (46). In early childhood, IGF-I output in preterm born children was shown to be correlated to weight and length (47).

We compared IGF-I levels of VLBW infants in early childhood with IGF-I levels of term AGA born children (Chapter 8). During the first 2 years of life, VLBW infants had higher IGF-I levels than term AGA born children. Based on earlier findings in mid-childhood, showing that 9-year-old preterm born children compared to term born children were shorter but had higher IGF-I levels, it was hypothesized that higher IGF-I levels indicate reduced sensitivity of the IGF-I receptors (46). In our study, however, we observed a decrease in difference in length of VLBW compared to term AGA children and a longitudinal relationship between IGF-I levels and growth during the first 2 years of life. Our findings might indicate that higher IGF-I levels in VLBW infants in early childhood have an important role in catch-up growth in length.

In conclusion, in early childhood the role of IGF-I is apparent from the longitudinal relation to growth in both VLBW and term born infants. Our study suggests that the higher IGF-I levels in VLBW infants during the first 2 years of life may have an important role in catch-up growth in length.

**Implications of the results**

The results of our studies have important implications for the life style and follow-up of VLBW infants. In the first place, because of the higher prevalence of some of the components of the metabolic syndrome, parents should be counselled about the risk of cardiovascular disease and given life style advices. As soon as VLBW children reach adolescence and adulthood, it is important that they become aware of the cardiovascular
risks associated with their preterm birth. Counselling should be aimed at prevention of the avoidable risk factors of cardiovascular disease, like obesity and smoking.

Healthcare professionals involved in the follow-up and care of VLBW children, like family-doctors and pediatricians, should also be aware of the metabolic consequences of preterm birth. They are responsible for counselling and evaluation of risk factors. Blood pressure should be measured on a regular basis during childhood and treatment should be started in case of hypertension. This should continue through adulthood and the information about the preterm birth should not get lost in the transition to adult care. It is important that the preterm birth is taken into account by adult care doctors as part of the assessment of the cardiovascular risk profile.

Future

Several aspects of the endocrine and metabolic consequences of preterm birth need further clarification and future studies are necessary.

For a good comparison between the activation of the HPG axis in VLBW infants and term born infants, future studies should include measurements of gonadotropins and testosterone/estradiol levels in serial urine samples of term born infants. Studies in older children and adults, both preterm and term born, are necessary to elucidate the consequences of the exaggerated activation of the HPG axis for puberty and reproductive function.

Insulin resistance plays a central role in the onset of the metabolic syndrome and our studies suggest that VLBW children already have reduced insulin sensitivity at 2 years corrected age. Future studies should measure insulin sensitivity in early childhood in VLBW children and term born controls. This could elucidate the pathophysiological mechanisms that lead to increased prevalence of metabolic syndrome components in VLBW children. Blood pressure of VLBW children has to be compared to blood pressure of term born children from our own population, because that is more reliable than the present comparison to published reference values.

Measurement of cortisol/cortisone ratio and several metabolic syndrome components, including blood pressure, in VLBW and term born children, at the same time points, in early and later childhood, is necessary to find out whether the higher cortisol/cortisone ratio persists in older children and to clarify the relationship between cortisol/cortisone ratio and metabolic parameters in both VLBW and term born children.

Continuous insulin treatment during the first 7 days of life could have long-term effects. Longer follow-up of the VLBW children participating in the NIRTURE trial has to show whether the possible metabolic advantages of early insulin treatment and the adverse effect on cortisol levels (and therefore possibly also on blood pressure) persist into later childhood and adulthood.
The role of IGF-I in growth of VLBW infants during childhood, its relation to catch-up growth and growth restriction and the significance of the higher IGF-I levels in preterm born children need further elucidation. Future studies should therefore include serial measurements of IGF-I levels from the early postnatal period until late childhood, in both VLBW infants and term born controls at the same time points. These serial IGF-I measurements during the period of catch-up growth could then clarify whether the higher IGF-I levels are necessary for catch-up, or may result from reduced sensitivity of the IGF-I receptors. Besides anthropometric data including body composition, nutritional information should also be collected during this period, to clarify the role of nutritional intake in growth restriction, catch-up growth and IGF-I levels.

In future studies, the samples required should preferably be taken by non-invasive methods, particularly by using urine and saliva samples. If hormone analysis requires the use of blood samples, assays requiring only minimal volumes of blood should preferably be used. This is especially important for children.

The knowledge obtained from our studies and future studies should lead to the development of methods to affect the adverse endocrine and metabolic consequences of preterm birth. Interventions will be aimed at optimizing growth, body composition and neurodevelopmental outcome and reducing the risk of insulin resistance and metabolic syndrome components. Interventions in the early postnatal period are probably most effective, as programming takes place in this period, which has long-term consequences.

According to our studies, continuous intravenous infusion of insulin during the first week of life could have long-term benefits for the metabolic profile. However, our studies also showed possible adverse effects (higher cortisol levels) of early insulin treatment. In view of these results and the absence of short-term clinical benefits (2), early insulin treatment according to the NIRTURE trial is presently not recommended as part of the standard neonatal care.

Low IGF-I levels play an important role in early postnatal growth restriction and therefore also in programming. Consequently, possible interventions should be targeted at increasing IGF-I levels in the early postnatal period. Optimizing nutrient intake only does not improve early postnatal growth and IGF-I levels. Because of the crucial role of IGF-I deficiency in growth restriction and programming, IGF-I suppletion in the early postnatal period could have long-term benefits. In addition, with IGF-I suppletion it is important to adapt nutritional intake to the higher obtained IGF-I levels, otherwise the excess of energy intake at that time might result in fat accumulation. In our opinion, in VLBW infants early postnatal IGF-I treatment with adapted nutritional intake could possibly reduce the risk of metabolic syndrome components in later life. Currently, clinical trials are conducted to evaluate the effects of early IGF-I treatment. Preliminary results show no serious adverse effects, particularly no hypoglycemia (48), and a reduced incidence of severe bronchopulmonary dysplasia (in preparation). Long-term follow-up of
the study population will be necessary to investigate the potential benefits of early IGF-I treatment on the development of insulin resistance and on other metabolic syndrome components in later life.

**FINAL CONCLUSIONS**

Being born with very-low-birth-weight affects the rest of life. Endocrine and metabolic consequences can already be detected in early childhood. Although the International Diabetes Federation stated that the metabolic syndrome as an entity should not be diagnosed in children younger than 10 years of age, it is important to realise that components of the metabolic syndrome are already present in early childhood in VLBW infants. This should be taken into account by healthcare professionals, parents and the VLBW children themselves during their entire lives. Reduced insulin sensitivity and programming of the HPA axis seem to play crucial roles in these later consequences of VLBW birth.

Continuous insulin treatment during the first week of life cannot be recommended from our studies in early childhood, but longer follow-up is necessary. IGF-I suppletion in the early postnatal period might be promising in reducing metabolic risks in later life in VLBW infants.

When designing new studies, always think critically about the type of specimen that will be taken for research purposes, especially in children. If available, non-invasive alternatives as urine and saliva are preferable above blood samples. Furthermore, for correct interpretation of study results, it is important to have reliable reference values, preferably based on age-matched controls from the same population.
REFERENCES


