Endocrine and metabolic consequences of preterm birth during early childhood

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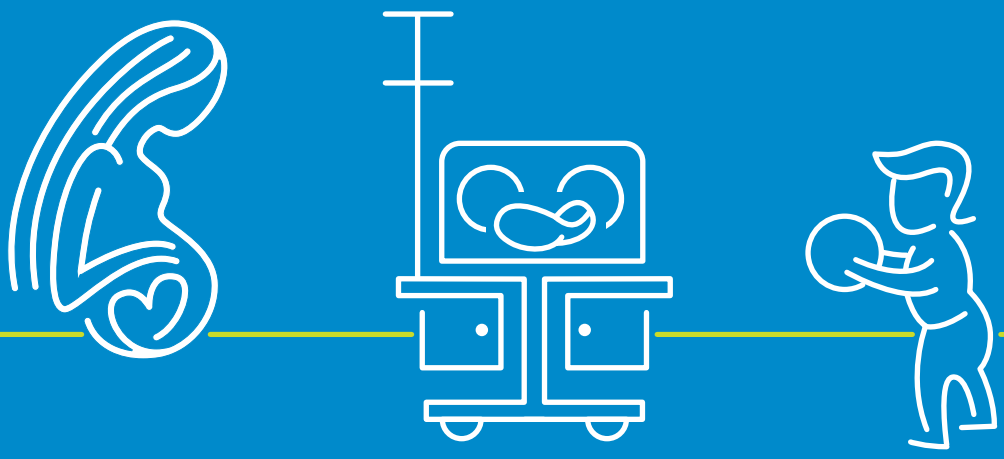

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promotoren: prof.dr. M.M. van Weissenbruch
prof.dr. H.N. Lafeber

Voor mijn ouders

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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.

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Chapter 2

Urine gonadotropin and testosterone levels in male very-low-birth-weight infants

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ABSTRACT

Background/Aims

The postnatal activation of the hypothalamic-pituitary-gonadal axis is more exaggerated in preterm than in full-term born infants and may be important for reproductive function. Our objective was to investigate this activation of the hypothalamic-pituitary-gonadal axis in male very-low-birth-weight infants.

Methods

Twenty-one very-low-birth-weight boys (gestational age 26.0-30.0 weeks), participating in the NIRTURE trial, were included. Gonadotropin and testosterone levels were measured in serial urine samples collected at 1 and 4 weeks postnatal age, at 32 weeks postmenstrual age, at expected date of delivery and at the corrected age of 3 and 6 months.

Results

Longitudinal analysis shows that after birth LH and FSH levels peak at a mean postnatal age of 1 to 4 weeks (mean postmenstrual age of 30 to 32 weeks) and decrease until 38 weeks postnatal age (corrected age of 6 months). Testosterone levels decrease with increasing age and this decrease is faster in infants receiving early insulin therapy.

Conclusions

Serial urine sampling for measurement of gonadotropin and testosterone levels provides accurate information about the postnatal activation of the hypothalamic-pituitary-gonadal axis in very-low-birth-weight boys. FSH and LH levels peak at 1 to 4 weeks of age. Insulin treatment causes faster decrease of testosterone levels.

INTRODUCTION

The postnatal activation of the hypothalamic-pituitary-gonadal axis that is observed during the first months of life, is considered as an important phase in the maturation of this axis. In boys, this activation has been shown to be associated to the development of the testes as well as the penis and the scrotum (1-5) and as a consequence the postnatal activation may be of importance for reproductive function in adult men.

The postnatal peak has been demonstrated in term as well as in preterm born boys (3, 6-17). In comparison with full-term boys, preterm born boys have higher levels of gonadotropins and testosterone during the first postnatal months (3, 10, 14). Data about the postnatal activity of the hypothalamic-pituitary-gonadal axis in preterm boys born at a gestational age less than 30 weeks are limited.

The pattern of hormone secretion in individual infants during this postnatal activation of the hypothalamic-pituitary-gonadal axis can accurately be described by serial hormone sampling. Most of the aforementioned studies in boys are cross-sectional and report serum levels of hormones. The use of urine samples makes it possible to collect serial measurements without the burden of frequent blood sampling and measurement of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in urine samples was previously shown to be reliable (18, 19).

The aim of the present study was to describe the postnatal activation of the hypothalamic-pituitary-testicular axis in male very-low-birth-weight (VLBW) infants by serial measurement of gonadotropin and testosterone levels in urine samples from birth to 9 months of age.

METHODS

Study population

The subjects were part of the Neonatal Insulin Replacement Therapy in Europe (NIRTURE) trial. This was an international multicenter randomized controlled trial investigating the role of early insulin therapy in VLBW infants (20). After informed parental consent was obtained, VLBW infants younger than 24 hours of age 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 included maternal diabetes and major congenital anomalies.

The 21 male infants participating in the NIRTURE trial in our neonatal intensive care unit were included in the present study. They had a mean gestational age of 28.2 weeks (range 26.0-30.0 weeks) and a mean birth weight of 1102 g (range 670-1460 g). Nine infants were assigned to the early-insulin group and 12 infants received standard neonatal

care. After discharge, all patients were followed in the outpatient clinic. Approval from the local ethics committee was obtained.

Urine samples

Urine samples were collected in a pediatric urine collection pouch at 1 and 4 weeks postnatal age, at 32 weeks postmenstrual age, at expected date of delivery and at the corrected age of 3 and 6 months. Samples were stored at -20 ºC until analysis.

FSH, LH and testosterone measurement

FSH and LH concentrations were measured by immunometric assays (Architect, Abbott Laboratories Diagnostics Division, Abbott Park, Illinois, USA), as validated and described earlier (19). For FSH lower limit of quantitation is 0.1 IU/l, intra-assay coefficients of variation are 5% at a level of 0.3 IU/l, 3% at 1.8 IU/l, 5.5 IU/l, 25 IU/l and 75 IU/l and inter-assay coefficients of variation are 6% at 5 IU/l and 5% at 18 IU/l. For LH lower limit of quantitation is 0.1 IU/l, intra-assay coefficient of variation is < 4% at 0.2 IU/l, 1.5 IU/l, 5 IU/l, 40 IU/l and 75 IU/l and inter-assay coefficients of variation are 7% at 4 IU/l and 6% at 23 IU/l.

After adding a sodiumacetate buffer (pH 4.8) and deuterated internal standard (testosterone-2,2,4,6,6-D_5; D_5T; obtained from CDN Isotopes, Pointe-Claire, Quebec, Canada), samples were hydrolyzed with helix pomatia juice (Pall Biosepra, Cergy-Saint-Christophe, France). The supernatant was injected to a Symbiosis online Solid Phase Extraction (SPE) system (Spark Holland, Emmen, The Netherlands). Online SPE with C18 cartridges (Spark Holland) was performed for further purification of the samples. Separation was achieved on a C18 analytical column (Kinetex, 2.1x75 mm, 2.6 µm particle size; Phenomenex, Utrecht, The Netherlands) by gradient elution, using 0.1% formic acid in water and 0.1% formic acid in acetonitrile. A Quattro Premier XE tandem mass spectrometer (Waters Corporation, Milford, Massachusetts, USA) with electrospray in positive mode was used for detection. Operating conditions were as follows: capillary voltage, 1.0 kV; cone voltage, 40 V; source temperature, 130 °C. Argon was used as collision gas. For each component (analyte and internal standard), two transitions were monitored: *m/z* 289>97 and *m/z* 289>109 for testosterone; *m/z* 294>100 and *m/z* 294>113 for D_5T. The first transitions in each set were used for quantification, the second transitions for confirmation. Data acquisition and processing were done with Masslynx 4.1 software (Waters Corporation). Total analysis time was 9 minutes. Linearity was tested by dilution between 3 and 43 nmol/l (R2 was 0.9992). Lower limit of quantitation was 1 nmol/l, intra-assay coefficient of variation for the whole range was < 7%. All analyses were performed in one run.

Gonadotropin and testosterone levels were corrected for creatinine levels. Creatinine concentrations were measured by the Jaffé method (Modular, Roche Diagnostics, Mannheim, Germany). Inter-assay coefficients of variation are 2.2% at 5.9 mmol/l and 1.7% at 12.5 mmol/l.

Statistical analysis

Statistical analyses were performed using the Statistical Package of Social Sciences software for Microsoft Windows version 17 (SPSS Inc., Chicago, Illinois, USA). At first FSH, LH and testosterone levels of infants treated with insulin were compared with hormone levels of infants receiving standard care. Because the distribution of hormone levels was skewed, a log transformation was performed before analysis. FSH, LH and testosterone levels were analyzed for postmenstrual age as well as for postnatal age, taking into account that both maturation and birth itself are important for activation of the hypothalamic-pituitary-gonadal axis. Longitudinal analyses were performed with generalized estimating equations, a method that takes into account that the repeated observations in one child are correlated. For gonadotropin levels below the limit of quantitation, a value of 0.01 U/l was used to discriminate these results from the values exactly on the limit of quantitation. Levels below the limit of quantitation were not corrected for creatinine. P values < 0.05 were considered as significant.

RESULTS

From our study population of 21 male VLBW infants, a total of 108 urine samples were collected for determination of LH, FSH and testosterone levels. At 1 week postnatal age, at expected date of delivery and at the corrected age of 3 months, urine samples were collected from all infants. At 4 weeks postnatal age, 32 weeks postmenstrual age and 6 months corrected age, samples were obtained from 19, 12 and 14 infants, respectively. In one sample collected at 6 months corrected age, only gonadotropin levels could be measured, because the volume was too small for all measurements.

Levels of LH, FSH and testosterone for postmenstrual age

The 108 urine samples were divided into six age groups, based on the postmenstrual age on the day of collection of the sample. The details of the age groups with mean postmenstrual age and number of samples as well as the median levels of FSH, LH and testosterone are shown in table 1. Because LH and FSH levels and patterns were not different between the early-insulin and standard care group, the groups were taken together for further analyses.

Figure 1 shows the median LH and FSH levels for increasing postmenstrual age. Longitudinal analysis showed that after birth LH levels significantly increased from 28 to 30 weeks postmenstrual age (corresponding to mean postnatal ages of 1 and 2 weeks) and significantly decreased from 32 until 66 weeks postmenstrual age (corrected age of 6 months) (corresponding to 4 until 38 weeks postnatal age); levels between 30 and 32 weeks postmenstrual age were not significantly different. FSH levels showed a

significant peak at 32 weeks postmenstrual age (4 weeks postnatal age) and thereafter significantly decreased until 66 weeks postmenstrual age (corrected age of 6 months or 38 weeks postnatal age).

Figure 2 shows the median testosterone levels with increasing postmenstrual age, for both the early-insulin and standard care group. In both groups longitudinal analysis showed that the observed peak is not significant; from 41 to 66 weeks postmenstrual age (13 to 38 weeks postnatal age), testosterone levels decreased significantly. Comparison between the early-insulin group and standard care group showed no significant differences between testosterone levels at all six mean postmenstrual ages, but the decrease from the highest levels was significantly faster in the early-insulin group.

Table 1. FSH, LH and testosterone levels for samples collected from six age groups based on postmenstrual age

Group		N	Postmenstrual age (weeks)	FSH (IU/mmol creatinine)	LH (IU/mmol creatinine)	Testosterone (nmol/mmol creatinine)
I	total	11	28.2 (27.0-29.3)	0.1 (0.01-1.3)	0.5 (0.01-3.6)	24.8 (7.1-102.0)
	standard	7	28.3 (27.0-29.3)	0.01 (0.01-0.9)	0.3 (0.01-2.4)	22.4 (10.9-102.0)
	insulin	4	28.0 (27.1-29.0)	0.5 (0.01-1.3)	1.0 (0.01-3.6)	32.1 (7.1-97.5)
II	total	17	30.4 (29.6-31.3)	0.6 (0.01-2.6)	1.3 (0.01-11.2)	31.9 (1.7-160.0)
	standard	9	30.5 (29.6-31.1)	0.7 (0.01-2.6)	1.3 (0.1-11.2)	31.1 (19.8-160.0)
	insulin	8	30.4 (29.6-31.3)	0.5 (0.01-2.5)	1.5 (0.01-8.2)	40.6 (1.7-93.8)
III	total	24	32.5 (31.4-34.0)	1.2 (0.2-5.6)	1.6 (0.09-11.2)	35.6 (2.4-87.3)
	standard	12	32.5 (31.7-34.0)	1.4 (0.2-5.6)	1.8 (0.2-11.2)	37.1 (24.9-87.3)
	insulin	12	32.5 (31.4-33.6)	0.9 (0.5-1.8)	1.3 (0.09-3.5)	30.3 (2.4-60.5)
IV	total	21	41.3 (39.6-44.7)	0.5 (0.2-1.4)	0.7 (0.01-5.7)	29.1 (2.5-61.7)
	standard	12	41.5 (39.6-44.7)	0.6 (0.2-1.3)	0.6 (0.09-5.7)	31.8 (11.9-61.7)
	insulin	9	41.0 (39.7-43.0)	0.5 (0.2-1.4)	0.7 (0.01-2.0)	20.9 (2.5-54.2)
V	total	21	53.7 (52.0-57.0)	0.2 (0.01-0.7)	0.09 (0.01-0.8)	11.1 (2.3-32.8)
	standard	12	53.7 (52.0-57.0)	0.2 (0.01-0.7)	0.1 (0.01-0.8)	11.6 (4.7-32.8)
	insulin	9	53.7 (52.0-57.0)	0.2 (0.01-0.5)	0.01 (0.01-0.4)	7.0 (2.3-26.2)
VI	total	14	66.0 (65.0-67.0)	0.03 (0.01-0.4)	0.01 (0.01-0.04)	2.7 (0.4-8.8)
	standard	7	66.1 (66.0-67.0)	0.01 (0.01-0.3)	0.01 (0.01-0.04)	3.1 (2.1-8.8)
	insulin	7	65.9 (65.0-67.0)	0.2 (0.01-0.4)	0.01 (0.01-0.03)	2.4 (0.4-4.9)

Hormone levels are expressed as median and range, postmenstrual age as mean and range. For each age group, results are given for all samples together, for samples of infants in the standard care group and for samples of infants in the early-insulin group. In all age groups, gonadotropin and testosterone levels did not differ significantly between the standard care and early-insulin group.

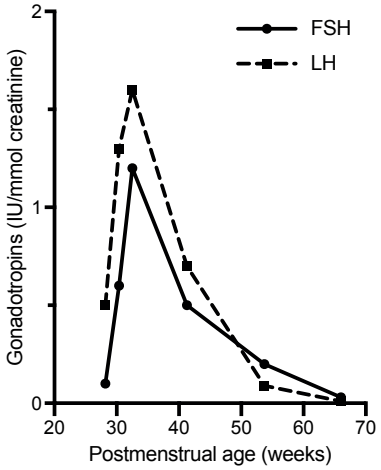

Figure 1. Median FSH and LH levels for samples collected from six age groups with increasing mean postmenstrual age.

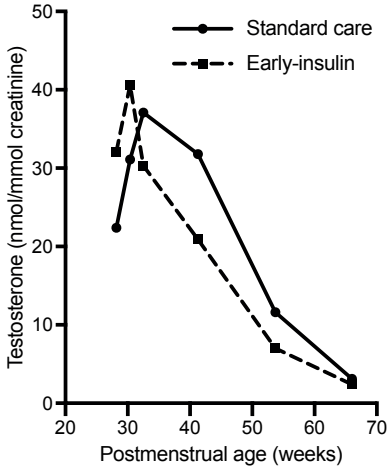

Figure 2. Median testosterone levels for samples collected from six age groups with increasing mean postmenstrual age and subdivided into early-insulin group and standard care group.

Table 2. FSH, LH and testosterone levels for samples collected from five age groups based on postnatal age

Group		N	Postnatal age (weeks)	FSH (IU/mmol creatinine)	LH (IU/mmol creatinine)	Testosterone (nmol/mmol creatinine)
I	total	28	1.5 (1.0-3.6)	0.4 (0.01-2.5)	1.0 (0.01-11.2)	40.5 (7.1-160.0)
	standard	15	1.4 (1.0-3.4)	0.2 (0.01-2.2)	1.0 (0.01-11.2)	33.3 (10.9-160.0)
	insulin	13	1.6 (1.0-3.6)	0.5 (0.01-2.5)	0.9 (0.01-8.2)	44.3 (7.1-97.5)
II	total	24	4.3 (4.0-5.9)	1.2 (0.2-5.6)	1.7 (0.2-11.2)	27.5 (1.7-87.3)
	standard	13	4.2 (4.0-5.7)	1.3 (0.2-5.6)	1.6 (0.2-11.2)	29.5 (19.8-87.3)
	insulin	11	4.3 (4.0-5.9)	0.9 (0.4-2.2)	1.8 (0.7-3,5)	21.2 (1.7-51.4)
III	total	21	13.1 (10.1-18.3)	0.5 (0.2-1.4)	0.7 (0.01-5.7)	29.1 (2.5-61.7)
	standard	12	13.3 (10.6-18.3)	0.6 (0.2-1.3)	0.6 (0.09-5.7)	31.8 (11.9-61.7)
	insulin	9	12.8 (10.1-15.0)	0.5 (0.2-1.4)	0.7 (0.01-2.0)	20.9 (2.5-54.2)
IV	total	21	25.5 (23.0-29.0)	0.2 (0.01-0.7)	0.09 (0.01-0.8)	11.1 (2.3-32.8)
	standard	12	25.6 (23.0-29.0)	0.2 (0.01-0.7)	0.1 (0.01-0.8)	11.6 (4.7-32.8)
	insulin	9	25.5 (23.7-27.6)	0.2 (0.01-0.5)	0.01 (0.01-0.4)	7.0 (2.3-26.2)
V	total	14	37.9 (35.7-40.0)	0.03 (0.01-0.4)	0.01 (0.01-0.04)	2.7 (0.4-8.8)
	standard	7	38.1 (36.6-40.0)	0.01 (0.01-0.3)	0.01 (0.01-0.04)	3.1 (2.1-8.8)
	insulin	7	37.8 (35.7-39.9)	0.2 (0.01-0.4)	0.01 (0.01-0.03)	2.4 (0.4-4.9)

Hormone levels are expressed as median and range, postnatal age as mean and range. For each age group, results are given for all samples together, for samples of infants in the standard care group and for samples of infants in the early-insulin group. In all age groups, gonadotropin and testosterone levels did not differ significantly between the standard care and early-insulin group.

Levels of LH, FSH and testosterone for postnatal age

For this analysis, the 108 urine samples were divided into five age groups, based on the postnatal age at the time of collection of the sample. Table 2 shows the details of these age groups with mean postnatal age, number of samples and median levels of FSH, LH and testosterone. LH and FSH levels and patterns were not different between the early-insulin and standard care group and the groups were taken together for further analyses.

Figure 3 shows the median LH and FSH levels for increasing postnatal age. Longitudinal analysis showed that after birth LH levels significantly decreased from 4 until 38 weeks postnatal age (corresponding to 32 until 66 weeks postmenstrual age); levels between 1 and 4 weeks postnatal age (29 and 32 weeks postmenstrual age) were not significantly different. FSH levels showed a significant peak at 4 weeks postnatal age (32 weeks postmenstrual age) and significantly decreased until 38 weeks postnatal age (66 weeks postmenstrual age or corrected age of 6 months).

Figure 4 shows the median testosterone levels with increasing postnatal age, for both the early-insulin and standard care group. From 1 to 4 weeks postnatal age (29 to 32 weeks postmenstrual age), there was a significant decrease only in the early-insulin group. Although the median testosterone level at 1 week postnatal age was higher in the early-insulin group, the difference with the standard care group was not significant. In both groups testosterone levels significantly decreased from 13 until 38 weeks postnatal age (41 until 66 weeks postmenstrual age). There were no significant differences in testosterone levels between the early-insulin and standard care group at all five mean postnatal ages.

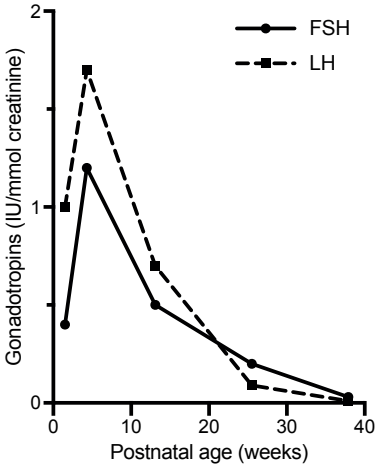

Figure 3. Median FSH and LH levels for samples collected from five age groups with increasing mean postnatal age.

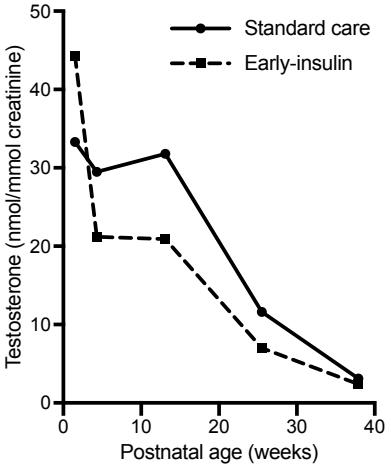

Figure 4. Median testosterone levels for samples collected from five age groups with increasing mean postnatal age and subdivided into early-insulin group and standard care group.

DISCUSSION

The present study provides an accurate description of the postnatal activation of the hypothalamic-pituitary-testicular axis in VLBW boys, based on serial measurements of gonadotropins and testosterone. The use of urine samples made it possible to collect these serial measurements without the disadvantages of serial blood sampling. FSH peaked at a mean postmenstrual age of 32 weeks (mean postnatal age of 4 weeks) and peak levels of LH were measured at mean postmenstrual ages of 30 and 32 weeks and mean postnatal ages of 1 and 4 weeks. Testosterone levels decreased with increasing age.

The first studies in preterm born male infants, performed more than 30 years ago, showed higher serum testosterone levels, a more prolonged increase with peak testosterone levels at 3 to 4 months of age and a slower decrease than in term born males (10, 14). The postnatal pituitary-gonadal activation is probably caused by loss of inhibitory feedback as a result of the disappearance of placental estrogens after birth. With increasing age, gonadotropin levels decline due to maturation of the inhibitory feedback system mediated by gonadal steroids and inhibin B (6, 10, 16, 17). The prolonged and more marked testicular activity in preterm born male infants is probably caused by the more immature state of the hypothalamic-pituitary-gonadal axis in preterms, which could be less sensitive for negative feedback by sex steroids and needs more time for full maturation of the inhibitory feedback system (10, 14).

The postnatal activation of the hypothalamic-pituitary-testicular axis is associated with an increase in numbers of Sertoli cells, Leydig cells and germ cells and testicular volume increases during infancy (1, 2, 5, 21). These testicular changes suggest that infancy is an important period for testicular development (22). The activation of the hypothalamic-pituitary-testicular axis with testosterone secretion in the first postnatal months is also important for a normal development of the external genitalia, suggested by lack of penile growth and involution of the scrotum in infants with hypogonadism (4). The importance of the exaggerated activation of the pituitary-testicular axis in preterm boys for reproductive function is still unclear.

Kuiri-Hanninen et al. (3) recently also used serial urine samples to study the postnatal activation of the hypothalamic-pituitary-gonadal axis in preterm born boys. They not only confirmed higher testosterone levels in preterm born boys compared to term born boys, but also showed that preterm born boys have higher levels of gonadotropins, which was not found in earlier studies (14, 23). They also found that the increased postnatal hypothalamic-pituitary-gonadal axis activation in preterm boys is associated with faster testicular and penile growth compared to full-term boys (3).

The study population of Kuiri-Hanninen et al. (3) is more heterogeneous than our study population and consists of preterm boys born at 24.7 to 36.6 weeks gestational age; less

than half was born at less than 32 weeks gestational age. We studied the hypothalamic-pituitary-gonadal axis using serial urine samples in preterm boys all born between 26 and 30 weeks gestational age. Concerning the gonadotropin levels, postnatal age at the peak levels and height of these levels in our study are in accordance with the results of Kuiri-Hanninen et al. (3). We did not find a significant peak in testosterone levels. Our analysis of testosterone levels for postmenstrual age shows highest levels at 30 and 32 weeks postmenstrual age; this is where peak levels are expected because the high LH levels at this age will probably cause Leydig cell stimulation. The absence of a significant peak in this analysis could be caused by the small number of infants. Our analysis of testosterone levels for postnatal age shows that levels are highest in the youngest age group (mean postnatal age of 1.5 weeks) and decrease with increasing age. This is in contrast with the results of Kuiri-Hanninen et al. (3), which showed peak testosterone levels at 1 month, and with the results of earlier studies (10, 14), where peak values were found at 3 to 4 months of age. This difference can be caused by the different study population as we studied only VLBW infants less than 30 weeks gestational age, whereas the other studies included only (10, 14), or also (3) older premature infants. It is also possible that we did not find a peak because of the small number of infants or the lack of data between 4 and 13 weeks postnatal age.

Testosterone levels were not significantly different between the early-insulin and standard care group, which could also be caused by the small number of patients. However, the decrease of testosterone levels was significantly faster in the early-insulin group. This could possibly be the result of a greater amount of adipose tissue and associated with this higher leptin levels in the infants treated with insulin. High leptin levels reduce testosterone production, probably by both a direct effect on the Leydig cells and by diminishing gonadotropin-releasing hormone secretion (24, 25). Ertl et al. (26) demonstrated an inverse relationship between leptin and testosterone levels during the first 5 weeks of life in male preterm born infants. In addition, as aromatase is mainly located in adipose tissue, increased aromatization of testosterone into estradiol could cause inhibition of gonadotropin release by estradiol in infants in the early-insulin group (24). In our study, we did not find evidence for a role of the amount of adipose tissue: there were no significant differences in body weight and total body fat, calculated according to Dauncey et al. (27) from skinfold thickness measurements and body dimensions, between infants in the early-insulin and standard care group (data not shown). We also did not find a difference in gonadotropins between the early-insulin and standard care group, which could have indicated an effect of leptin or increased aromatization. The absence of these differences could be caused by the small number of infants. The effect of insulin on testosterone levels could also be mediated by its effect on sex hormone-binding globulin as the negative correlation between insulin and sex hormone-binding globulin is already present at birth (28).

Our study is limited by the small number of infants and the lack of data from term born male infants. The results have to be confirmed in a larger group of VLBW infants. To make a good comparison with the hypothalamic-pituitary-testicular activation in term born boys, a study should be performed using serial urine samples of term born male infants to measure gonadotropins and testosterone using the same assays.

In conclusion, by using urine samples for serial measurements of gonadotropins and testosterone, we were able to get an accurate impression of the postnatal activation of the hypothalamic-pituitary-testicular axis in VLBW boys. Levels of LH and FSH peak at a mean postnatal age of 1 to 4 weeks (mean postmenstrual age of 30 to 32 weeks). Testosterone levels do not show a significant peak and decrease with increasing age; this decrease is faster in infants receiving early insulin therapy compared to those receiving standard care.

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Chapter 3

Urine gonadotropin and estradiol levels in female very-low-birth-weight infants

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ABSTRACT

Background

The postnatal activation of the hypothalamic-pituitary-gonadal axis is more exaggerated in preterm than in full-term born infants and may be important for future reproductive function.

Aim

The objective of this study was to investigate the postnatal activation of the hypothalamic-pituitary-gonadal axis in female very-low-birth-weight infants.

Study design

We performed serial measurements of gonadotropin and estradiol levels in urine samples of female very-low-birth-weight infants collected at 1 and 4 weeks postnatal age, at 32 weeks postmenstrual age, at expected date of delivery and at the corrected age of 3 and 6 months.

Subjects

Twenty-two very-low-birth-weight infants (gestational age 25.4-30.1 weeks), participating in the Neonatal Insulin Replacement Therapy in Europe trial, were included in this study.

Outcome measures

Gonadotropin and estradiol levels were measured in serial urine samples.

Results

Longitudinal analysis shows that after birth FSH and LH levels increase until 32 weeks postmenstrual age (4 weeks postnatal age) and then decrease until 3 months corrected age (26 weeks postnatal age). Estradiol levels decrease from 28 weeks postmenstrual age (1 week postnatal age) until 6 months corrected age (39 weeks postnatal age).

Conclusions

Serial urine sampling for measurement of gonadotropin and estradiol levels provides an accurate description of the postnatal activation of the hypothalamic-pituitary-gonadal axis in very-low-birth-weight girls. Levels of FSH and LH peak at a mean postmenstrual age of 32 weeks (postnatal age of 4 weeks), whereas estradiol levels are highest shortly after birth.

INTRODUCTION

The postnatal activation of the hypothalamic-pituitary-gonadal axis that is observed during the first months of life, is considered as an important phase in the maturation of the gonads and may play a significant role in the development of reproductive function. However, the mechanisms that underlie this activation and the exact importance for fertility are not well understood yet.

Several studies describe this postnatal activation in term born infants (1-6). In preterm born infants, the postnatal activation of the hypothalamic-pituitary-gonadal axis seems to be more exaggerated than in full-term born infants (7-11). Data about the postnatal activity of the hypothalamic-pituitary-gonadal axis in preterm infants born at a gestational age less than 30 weeks are limited.

Most of the studies in both term and preterm born infants have a cross-sectional design and report serum levels of gonadotropins and sex steroids. Serial serum samples give accurate information about the pattern of hormone secretion in individuals. However, serial blood sampling in infants also has major disadvantages, including the pain caused by the puncture and the risk of iatrogenic anemia, especially in very-low-birth-weight (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. Urine sampling does not have these disadvantages and both gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH) can be reliably measured in urine samples (12, 13).

It was hypothesized that serial gonadotropin levels in combination with serial estradiol levels measured in urine during the first postnatal months, can be used to describe the postnatal activation of the hypothalamic-pituitary-ovarian axis in female VLBW infants. Therefore the aim of the present study was to investigate gonadotropin and estradiol secretion in serial urine samples of female VLBW infants from birth to 9 months of age.

METHODS

Study population

The subjects were part of the Neonatal Insulin Replacement Therapy in Europe (NIRTURE) trial. This was an international multicenter randomized controlled trial investigating the role of early insulin therapy in VLBW infants (14). After informed parental consent was obtained, VLBW infants younger than 24 hours of age 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 included maternal diabetes and major congenital anomalies.

The 22 female infants participating in the NIRTURE trial in our neonatal intensive care unit were included in the present study. They had a mean gestational age of 27.6 weeks (range 25.4-30.1 weeks) and a mean birth weight of 998 g (range 540-1415 g). Ten infants were assigned to the early-insulin group and 12 infants received standard neonatal care. After discharge, all patients were followed in the outpatient clinic. Approval from the local ethics committee was obtained.

Urine samples

Urine samples were collected in a pediatric urine collection pouch at 1 and 4 weeks postnatal age, at 32 weeks postmenstrual age, at expected date of delivery and at the corrected age of 3 and 6 months. Samples were stored at -20 ^{o}C until analysis.

FSH, LH and estradiol measurement

FSH and LH concentrations were measured by immunometric assays (Architect, Abbott Laboratories Diagnostics Division, Abbott Park, Illinois, USA). For FSH lower limit of quantitation is 0.11 IU/l, intra-assay coefficient of variation is 3% at 5.5 IU/l, 25 IU/l and 75 IU/l and inter-assay coefficients of variation are 6% at 5 IU/l and 5% at 18 IU/l. For LH lower limit of quantitation is 0.1 IU/l, intra-assay coefficient of variation is 3% at 5 IU/l, 40 IU/l and 75 IU/l and inter-assay coefficients of variation are 7% at 4 IU/l and 6% at 23 IU/l.

After hydrolysis with helix pomatia juice (Pall Biosepra, Cergy-Saint-Christophe, France) and extraction with diethyl ether, urinary estradiol concentration was measured by competitive immunoassay (Architect, Abbott Laboratories Diagnostics Division, Abbott Park, Illinois, USA), as also applied by Peper et al. (15). Intra-assay coefficients of variation are 9%, 3% and 4% at a level of 150, 1400 and 9000 pmol/l, respectively and inter-assay coefficient of variation is 10% for the whole range.

Gonadotropin and estradiol levels were corrected for creatinine levels. Creatinine concentrations were measured by the Jaffé method (Modular, Roche Diagnostics, Mannheim, Germany). Inter-assay coefficients of variation are 2.2% at 5.9 mmol/l and 1.7% at 12.5 mmol/l.

Statistical analysis

Statistical analyses were performed using the Statistical Package of Social Sciences software for Microsoft Windows version 17 (SPSS Inc., Chicago, Illinois, USA). At first FSH, LH and estradiol levels of infants treated with insulin were compared with hormone levels of infants receiving standard care. Because the distribution of hormone levels was skewed, a log transformation was performed before analysis. To distinguish the role of birth from that of maturation in the activation of the hypothalamic-pituitary-gonadal axis, FSH, LH and estradiol levels were analyzed in relation to postmenstrual age as well as to postnatal age. If the influence of birth itself is most important for the activation, peak hormone levels are expected to be found at comparable postnatal ages as in term

born infants; if, on the other hand, the degree of maturation comparable to term born infants is required for the activation of the hypothalamic-pituitary-gonadal axis, then peak hormone levels are expected to be found at comparable postmenstrual ages (ages corrected for prematurity). Longitudinal analyses were performed with generalized estimating equations, a method that takes into account that the repeated observations in one child are correlated. For gonadotropin levels below the limit of detection, a value of 0.01 U/l was used. P values < 0.05 were considered as significant.

RESULTS

From our study population of 22 female VLBW infants, a total of 94 urine samples were collected for determination of FSH, LH and estradiol levels.

Levels of FSH, LH and estradiol for postmenstrual age

The urine samples were divided into six age groups, based on the postmenstrual age on the day of collection of the sample. The details of the age groups with mean postmenstrual age and number of samples as well as the median levels of FSH, LH and estradiol are shown in table 1. Because hormone levels were not different between the early-insulin and standard care group, the groups were taken together for further analyses.

Longitudinal analysis showed that after birth FSH levels significantly increased from 28 weeks postmenstrual age to 32 weeks postmenstrual age. Thereafter FSH levels significantly decreased until 53 weeks postmenstrual age (corrected age of 3 months) (Table 1 and Figure 1). LH levels significantly increased from 30 weeks postmenstrual age to 32 weeks postmenstrual age. Thereafter LH levels significantly decreased until 53 weeks postmenstrual age (corrected age of 3 months) (Table 1 and Figure 1). Estradiol levels significantly decreased from 28 weeks postmenstrual age until 66 weeks postmenstrual age (corrected age of 6 months) (Table 1 and Figure 2).

Levels of FSH, LH and estradiol for postnatal age

For this analysis, urine samples were divided into five age groups, based on the postnatal age at the time of collection of the sample. Table 2 shows the details of these age groups with mean postnatal age, number of samples and median levels of FSH, LH and estradiol. Hormone levels were not different between the early-insulin and standard care group and the groups were taken together for further analyses.

Longitudinal analysis showed that after birth both FSH and LH levels significantly increased from 1 week to 4 weeks postnatal age. Thereafter FSH and LH levels significantly decreased until 26 weeks postnatal age (Table 2 and Figure 3). Estradiol levels significantly decreased from 1 week postnatal age until 39 weeks postnatal age (Table 2 and Figure 4).

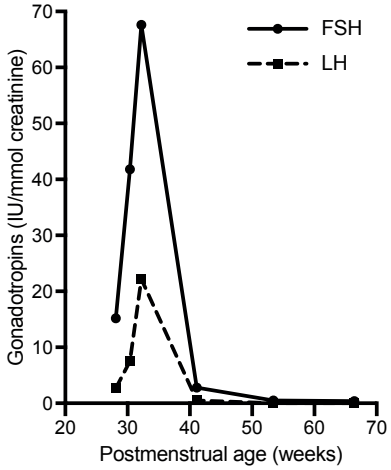

Figure 1. Median FSH and LH levels for samples collected from six age groups with increasing mean postmenstrual age.

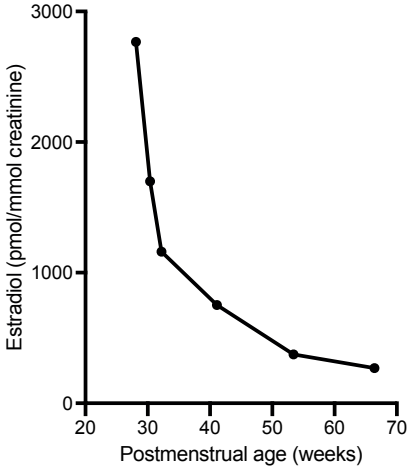

Figure 2. Median estradiol levels for samples collected from six age groups with increasing mean postmenstrual age.

Table 1. FSH, LH and estradiol levels for samples collected from six age groups based on postmenstrual age

Group		N	Postmenstrual age (weeks)	FSH (IU/mmol creatinine)	LH (IU/mmol creatinine)	Estradiol (pmol/mmol creatinine)
I	total	15	28.1 (26.4-29.1)	15.2 (0.7-86.0)	2.7 (0.01-30.8)	2767 (1343-12629)
	standard	6	28.3 (27.4-29.1)	8.6 (1.4-86.0)	0.9 (0.1-17.2)	2522 (1401-3820)
	insulin	9	27.9 (26.4-29.1)	18.4 (0.7-61.7)	6.7 (0.01-30.8)	3893 (1343-12629)
II	total	11	30.4 (29.4-31.1)	41.8 (2.5-258.2)	7.5 (0.01-49.3)	1700 (1053-4020)
	standard	7	30.6 (29.4-31.1)	23.0 (2.5-91.0)	6.7 (0.01-10.7)	1700 (1053-4020)
	insulin	4	30.0 (29.4-30.6)	93.0 (7.7-258.2)	16.4 (1.9-49.3)	1698 (1152-2165)
III	total	14	32.2 (31.6-33.6)	67.6 (25.1-183.5)	22.2 (0.9-65.4)	1160 (659-2873)
	standard	10	32.1 (31.6-33.4)	64.4 (25.1-183.5)	17.5 (0.9-65.4)	1268 (659-2855)
	insulin	4	32.5 (32.0-33.6)	98.8 (53.9-141.8)	23.6 (13.2-29.1)	955 (862-2873)
IV	total	21	41.1 (39.6-44.7)	2.8 (0.6-33.7)	0.5 (0.01-10.7)	752 (200-1952)
	standard	12	41.0 (39.6-42.1)	2.7 (0.6-20.7)	0.08 (0.01-10.7)	791 (200-1740)
	insulin	9	41.2 (40.0-44.7)	7.3 (0.8-33.7)	1.1 (0.07-4.3)	534 (292-1952)
V	total	20	53.4 (52.0-56.0)	0.5 (0.01-2.1)	0.01 (0.01-0.2)	374 (155-697)
	standard	11	53.5 (53.0-56.0)	0.3 (0.01-1.1)	0.01 (0.01-0.01)	348 (155-697)
	insulin	9	53.1 (52.0-54.0)	0.7 (0.01-2.1)	0.01 (0.01-0.2)	406 (240-676)
VI	total	13	66.4 (65.0-68.0)	0.4 (0.03-3.8)	0.01 (0.01-0.1)	269 (116-469)
	standard	9	66.4 (65.0-68.0)	0.4 (0.03-3.8)	0.01 (0.01-0.1)	265 (116-460)
	insulin	4	66.3 (66.0-67.0)	0.8 (0.4-2.2)	0.01 (0.01-0.01)	307 (220-469)

Hormone levels are expressed as median and range, postmenstrual age as mean and range. For each age group, results are given for all samples together, for samples of infants in the standard care group and for samples of infants in the early-insulin group. In all age groups, gonadotropin and estradiol levels did not differ significantly between the standard care and early-insulin group.

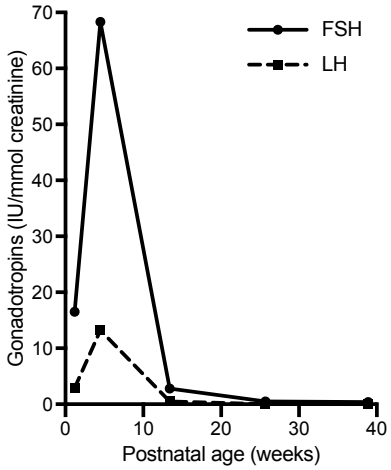

Figure 3. Median FSH and LH levels for samples collected from five age groups with increasing mean postnatal age.

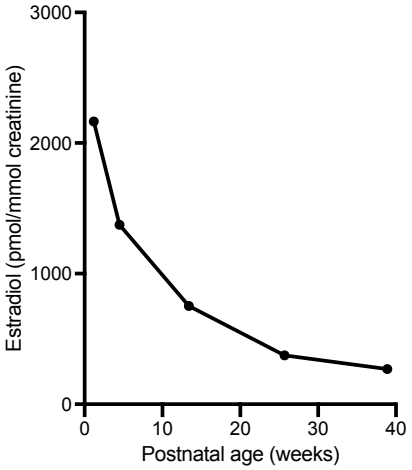

Figure 4. Median estradiol levels for samples collected from five age groups with increasing mean postnatal age.

Table 2. FSH, LH and estradiol levels for samples collected from five age groups based on postnatal age

Group		N	Postnatal age (weeks)	FSH (IU/mmol creatinine)	LH (IU/mmol creatinine)	Estradiol (pmol/mmol creatinine)
I	total	23	1.2 (1.0-3.6)	16.5 (0.7-86.0)	2.9 (0.01-30.8)	2165 (1053-12629)
	standard	13	1.4 (1.0-3.6)	15.2 (1.4-86.0)	2.9 (0.01-24.1)	1700 (1053-4020)
	insulin	10	1.0 (1.0-1.0)	17.5 (0.7-61.7)	4.7 (0.01-30.8)	3775 (1343-12629)
II	total	17	4.5 (4.0-6.6)	68.3 (7.7-258.2)	13.2 (0.9-65.4)	1374 (659-3210)
	standard	10	4.6 (4.0-5.7)	64.4 (7.7-183.5)	11.6 (0.9-65.4)	1592 (659-3210)
	insulin	7	4.4 (4.0-6.6)	129.3 (53.9-258.2)	24.4 (8.3-49.3)	1152 (862-2873)
III	total	21	13.4 (10.3-18.3)	2.8 (0.6-33.7)	0.5 (0.01-10.7)	752 (200-1952)
	standard	12	12.9 (10.3-15.6)	2.7 (0.6-20.7)	0.08 (0.01-10.7)	791 (200-1740)
	insulin	9	14.1 (10.6-18.3)	7.3 (0.8-33.7)	1.1 (0.07-4.3)	534 (292-1952)
IV	total	20	25.7 (23.4-28.3)	0.5 (0.01-2.1)	0.01 (0.01-0.2)	374 (155-697)
	standard	11	25.4 (23.6-27.7)	0.3 (0.01-1.1)	0.01 (0.01-0.01)	348 (155-697)
	insulin	9	26.0 (23.4-28.3)	0.7 (0.01-2.1)	0.01 (0.01-0.2)	406 (240-676)
V	total	13	38.9 (35.0-41.6)	0.4 (0.03-3.8)	0.01 (0.01-0.1)	269 (116-469)
	standard	9	38.7 (35.0-40.9)	0.4 (0.03-3.8)	0.01 (0.01-0.1)	265 (116-460)
	insulin	4	39.4 (37.9-41.6)	0.8 (0.4-2.2)	0.01 (0.01-0.01)	307 (220-469)

Hormone levels are expressed as median and range, postnatal age as mean and range. For each age group, results are given for all samples together, for samples of infants in the standard care group and for samples of infants in the early-insulin group. In all age groups, gonadotropin and estradiol levels did not differ significantly between the standard care and early-insulin group.

DISCUSSION

In the present study, we confirm postnatal activation of the pituitary-ovarian axis in preterm born female infants. Moreover, by serial measurement of gonadotropins and estradiol using urine samples we were able to get an accurate impression of the postnatal activation of the pituitary-gonadal axis in VLBW girls.

Previous studies in female term born infants indicated measurable serum gonadotropin levels with peak concentrations during the first months of life, followed by a decline to pre-pubertal levels during the first years of life: FSH peak values were measured in the first 3 months of life and levels stayed above those of older pre-pubertal children until 4 years of age; LH values were always lower than FSH values, reached maximum levels around 1 month of age and were in the normal pre-pubertal range after 4 months of age (2, 4, 5).

In contrast to gonadotropin levels, estradiol levels are high in cord serum followed by a rapid fall after birth due to disappearance of placental estrogens, reaching pre-pubertal levels 4 months after birth (6). A possible explanation for the postnatal pituitary-gonadal activation is that this fall in estradiol concentration causes the rise in gonadotropin concentrations by loss of inhibitory feedback. In turn, high levels of gonadotropins result in ovarian stimulation and production of estradiol. With maturation of the inhibitory feedback system by gonadal steroids, the gonadotropin concentrations decline again with increasing age (5, 6). The exact importance of this postnatal activation for normal development and function of the ovaries and future fertility is still unclear.

In preterm born female infants, serum gonadotropin levels are higher during the first 10 weeks of life compared to term born female infants, with FSH levels 10-20 times higher and LH levels 3-4 times higher (10). Peak FSH and LH levels are reached between 11 and 30 postnatal days and the peak is more marked and prolonged in preterm compared to term born female infants (9). To explain these differences between preterm and term born female infants, it was suggested that the higher gonadotropin levels in premature born girls were probably caused by the immature state of the hypothalamic-pituitary-gonadal axis, which could be less sensitive for negative feedback by the postnatal decline in estradiol levels than in term born infants (10). Measurement of estradiol levels in 3-month-old girls shows that preterm born girls also have higher estradiol levels than term born girls (16). The relevance of the exaggerated activation of the pituitary-ovarian axis in preterm girls for ovarian development and reproductive function is not yet known.

Cross-sectional data of serum gonadotropin levels during the first 6 weeks of life collected from extremely premature infants born between 24 and 29 weeks gestational age show very variable FSH and LH levels in female infants without any obvious peak

level during this period (17). As we used urine samples in the present study, we were not limited by the disadvantages of serial serum sampling.

Kuiri-Hanninen et al. (8, 11) recently reported gonadotropin levels in serial urine samples of full-term and preterm born infant boys and girls. In boys they demonstrated increased postnatal hypothalamic-pituitary-gonadal axis activation associated with faster testicular and penile growth in preterm compared to full-term born infants (8). In addition, they found that in term as well as preterm girls FSH levels were highest at 1 month of age and resulted in transient ovarian stimulation. As they observed a delay in ovarian folliculogenesis in preterm compared to full-term born girls, it was speculated that insufficient inhibitory feedback by ovarian inhibins and estrogens could cause the higher and more prolonged FSH peak in preterm girls (11). Kuiri-Hanninen et al. (11) studied preterm girls with a wide range of gestational ages (24.7 to 36.7 weeks) and birth weights (530 to 2720 g). We studied the hypothalamic-pituitary-gonadal axis using serial urine samples in preterm girls all born between 25.4 and 30.1 weeks with birth weights less than 1500 g.

In our study, both FSH and LH show a peak at a mean postmenstrual age of 32 weeks, corresponding to a mean postnatal age of 4 weeks. As peak hormone levels were measured at a comparable postnatal age as in previous studies in term born female infants (2, 4, 5), this suggests that birth itself plays a crucial role in the activation of the hypothalamic-pituitary-gonadal axis. At 3 and 6 months corrected age FSH levels are low, but still measurable in most infants, while at that time LH levels are immeasurable in most infants. This is in accordance with the results of Winter et al. (5), who described persistence of higher FSH levels until 4 years of age. The absence of an obvious peak in gonadotropin concentrations in an earlier study of Greaves and Hunt et al. (17) in extremely premature female infants may have been caused by the cross-sectional design of that study. Cross-sectional studies are more limited in detecting patterns of hormone secretion than longitudinal studies.

As far as we know estradiol levels have not been measured serially in preterm born girls in previous studies. Estradiol levels are highest in the youngest age group (mean postmenstrual age of 28 weeks) and decrease with increasing age. The decrease after birth is caused by the disappearance of placental estrogens. Peak gonadotropin levels are preceded by a decrease in estradiol levels, supporting the hypothesis that the rise in gonadotropin concentrations is caused by a decrease of inhibitory feedback by estradiol. Kuiri-Hanninen et al. (11) showed ovarian stimulation following the postnatal FSH peak, so it is probable that rising gonadotropin levels stimulate the ovarian production of estradiol. This stimulation is maximal around 4 weeks of age and subsequently decreases. Despite this ovarian stimulation estradiol levels in our study did not show a peak. It is possible that we did not find this peak because of insufficient data between days 7 and 28 or the small number of infants. On the other hand, Winter et al. (6) studied

postnatal estradiol levels in term born female infants and did not find a peak either; they suggested that the considerable variability in estradiol levels in female infants could be caused by growth and regression of ovarian follicles.

Limitations of our study are the small number of infants and the lack of data from term born female infants. The results have to be confirmed in a larger group of VLBW infants. To make a good comparison with the patterns and values of gonadotropins and estradiol in term born girls, a study should be performed with use of the same assays to measure levels of gonadotropins and estradiol in serial urine samples of term born female infants.

In conclusion, by using urine samples we were able to collect serial measurements of gonadotropin and estradiol levels in female VLBW infants without the burden of frequent blood sampling. This provides an accurate description of the postnatal activation of the hypothalamic-pituitary-gonadal axis in VLBW girls. Levels of FSH and LH peak at a mean postmenstrual age of 32 weeks (postnatal age of 4 weeks) and estradiol levels are highest shortly after birth.

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Chapter 4

Components of the metabolic syndrome in early childhood in very-low-birth-weight infants

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ABSTRACT

Background/Aims

Term small-for-gestational-age and preterm born infants have an increased prevalence of metabolic syndrome components already in childhood. Data in very-low-birth-weight (VLBW) children are limited. We investigated the prevalence of metabolic syndrome components in VLBW infants at 2 years corrected age.

Methods

We included 38 children, participating in the Neonatal Insulin Replacement Therapy in Europe (NIRTURE) trial, a randomized controlled trial of early insulin therapy in VLBW infants. Metabolic syndrome components were defined as: body mass index (BMI) SDS > 2; blood pressure (systolic and/or diastolic) ≥ 90th percentile; triglycerides ≥ 0.98 mmol/l; high-density lipoprotein (HDL) cholesterol ≤ 1.03 mmol/l; glucose ≥ 5.6 mmol/l.

Results

Two children (5%) had three metabolic syndrome components, 13 children (34%) had two components and 11 children (29%) one component. 63% had raised blood pressure (prevalence higher in boys), 32% low HDL cholesterol and 30% high triglycerides (prevalence lower in early-insulin group). In children with BMI SDS < 0, insulin-treated children had higher HDL cholesterol than children with standard care. Systolic blood pressure was correlated with growth between term and 2 years corrected age.

Conclusions

VLBW infants already have a high prevalence of metabolic syndrome components at 2 years corrected age. Early insulin treatment could have long-term benefits for some of these components.

INTRODUCTION

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. 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 later life, not only in adulthood (1-5), but already in childhood (6-13). Studies in very-low-birth-weight (VLBW) infants show increased blood pressure and insulin resistance in adulthood (14-17). Data about the prevalence of the components of the metabolic syndrome in VLBW infants during childhood are very limited.

The aim of the present study was to investigate the prevalence of the components of the metabolic syndrome in VLBW infants at the corrected age of 2 years. As the subjects were part of the Neonatal Insulin Replacement Therapy in Europe (NIRTURE) trial, our second aim was to evaluate the effect of early insulin therapy on the components of the metabolic syndrome at the corrected age of 2 years.

METHODS

Study population

The subjects were part of the NIRTURE trial, an international multicenter randomized controlled trial investigating the role of early insulin therapy in VLBW infants (18). After written informed consent was obtained, 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 included maternal diabetes and major congenital anomalies. All infants participating in the NIRTURE trial in our neonatal intensive care unit were eligible for the present study. Therefore the sample size of the present study was determined by the number of infants we included in the NIRTURE trial. After discharge, all patients were followed in the outpatient clinic with visits at expected date of delivery and at the corrected ages of 3 months, 6 months, 1 year and 2 years. Approval from the local ethics committee was obtained.

Data collection

At the corrected age of 2 years, anthropometry according to Dauncey et al. (19) was performed by a trained research nurse. Body weight was measured using an electronic scale to the nearest 0.1 kg, standing height was measured to the nearest 0.1 cm and all lengths and circumferences were measured using a measuring tape to the nearest

0.1 cm. Body mass index (BMI) was then calculated. Standard deviation scores (SDS) of weight, height and BMI were calculated according to Dutch references (20, 21). Blood pressure was measured using an appropriately sized cuff and automated blood pressure measuring device (Dinamap, Critikon, Tampa, Florida, USA); the mean value of two measurements was used for analysis. A blood sample for measurement of glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides was taken after a fasting period of at least 4 hours.

Assays

Total cholesterol, HDL cholesterol and triglycerides were measured by enzymatic colorimetric assay (CHOD-PAP, HDL-C plus and GPO-PAP, respectively; Modular Analytics, Roche diagnostics, Mannheim, Germany). Inter-assay coefficient of variation is 1.9% at both 3.5 mmol/l and 7.1 mmol/l for total cholesterol, 2.9% at 1.0 mmol/l and 2.8% at 2.4 mmol/l for HDL cholesterol and 3.0% at 1.1 mmol/l and 2.3% at 1.9 mmol/l for triglycerides.

Glucose concentrations were measured by the hexokinase method (Modular Analytics, Roche diagnostics, Mannheim, Germany). Inter-assay coefficients of variation are 2.0% at 4.8 mmol/l and 1.8% at 19.8 mmol/l

Definitions

There is no standard definition of the metabolic syndrome in children. Ford and Li (22) reviewed all previously used definitions; most common is the definition of Cook et al. (23), based on the criteria of the National Cholesterol Education Program (NCEP), Adult Treatment Panel III for the metabolic syndrome in adults (24). According to this definition, the metabolic syndrome is diagnosed in adolescents in the presence of at least 3 of the following 5 criteria: waist circumference ≥ 90th percentile; blood pressure (systolic and/or diastolic) ≥ 90th percentile; triglycerides ≥ 1.24 mmol/l (110 mg/dl); HDL cholesterol ≤ 1.03 mmol/l (40 mg/dl); fasting glucose ≥ 6.1 mmol/l (110 mg/dl) (23). According to the International Diabetes Federation (IDF) consensus report, the metabolic syndrome as an entity should not be diagnosed in children younger than 10 years of age (25). Therefore, we investigated the prevalence of the components of the metabolic syndrome and not the prevalence of the metabolic syndrome itself.

We used the definition of Cook et al. (23) as the basis for the definitions of the components of the metabolic syndrome in the 2-year-old children in the present study. Because waist circumference measurements were not part of the routine anthropometry, we used the definition of obesity of Weiss et al. (26): BMI SDS > 2 for age and sex. For blood pressure we used the percentiles for age, sex and height of the National High Blood Pressure Education Program Working Group (27). As cut-off values for lipids, Cook et al. (23) used the midpoint value in the borderline low range (between 5th and 25th percentile) for

HDL cholesterol and in the borderline high range (between 75th and 95th percentile) for triglycerides, based on the NCEP Report of the Expert Panel on Blood Cholesterol Levels in Children and Adolescents (28). As the HDL cholesterol borderline range applies to all ages, we also used the cut-off value of 1.03 mmol/l. The triglycerides borderline range is lower in children aged less than 10 years (0.85-1.12 mmol/l), so we used the midpoint value in this range (0.98 mmol/l (87 mg/dl)) as cut-off value for triglycerides. For fasting glucose we used a lower level of 5.6 mmol/l (100 mg/dl), according to the IDF definition of the metabolic syndrome in children and adolescents (25). We did not use the complete IDF definition as the basis for our definitions, as they use adult levels for all components except for obesity; their definition of obesity is the same as that of Cook et al. (23).

Statistical analysis

Statistical analyses were performed using the Statistical Package of Social Sciences software for Microsoft Windows version 19 (SPSS Inc., Chicago, Illinois, USA). Differences between subgroups were evaluated using Student's t-test and Chi-Square tests. Bivariate correlation analysis was performed to study the relation between parameters of growth and components of the metabolic syndrome. P values < 0.05 were considered as significant.

RESULTS

During the inclusion period of the NIRTURE trial in our neonatal intensive care unit (21 months), 165 VLBW infants were admitted and the parents of 69 infants were approached regarding participation in the study. The most common reasons for not approaching parents were infants not requiring intensive care or no opportunity to obtain informed consent within the first 24 hours after birth. In our unit, 47 VLBW infants participated in the NIRTURE trial. Five infants died and 2 children were lost to follow-up. At the corrected age of 2 years, 40 children visited our outpatient clinic. Two children were excluded because the parents refused blood sampling, 38 children were included in the present study. They had a mean gestational age of 27.9 weeks (range 25.4-30.1 weeks) and a mean birth weight of 1059 g (range 680-1460 g; range birth weight SDS -1.8 to 1.3). Three children (8%) were SGA, defined as a birth weight below the 10th percentile. Most infants (n = 23) were Caucasian, 10 were Black, 3 Moroccan and 2 Asian. Highest level of education completed by either parent, as an indicator of socioeconomic status, was low (primary school, low occupational training) in 3 children, medium (high school, medium occupational training) in 16 children and high (high occupational training, university) in 19 children. Thirty-six infants received antenatal steroids and 2 received postnatal steroids. Seventeen infants (9 male/8 female) were assigned to the early-

insulin group and 21 infants (10 male/11 female) received standard neonatal care. Seven children developed bronchopulmonary dysplasia, defined as the need for supplemental oxygen at 36 weeks postmenstrual age. At the corrected age of 2 years, 2 children were developmentally delayed and 3 had cerebral palsy.

Table 1. Characteristics of the VLBW children at birth and at the corrected age of 2 years

	Girls (n=19)	**Boys (n=19)**	**Standard care (n=21) (10M/11F)**	**Early-insulin (n=17) (9M/8F)**
At birth				
Gestational age (wk)	27.7 ± 1.4	28.0 ± 1.3	28.0 ± 1.3	27.7 ± 1.4
Weight (g)	1007 ± 240	1112 ± 205	1054 ± 231	1066 ± 226
Weight SDS	-0.1 ± 0.8	0.0 ± 0.8	-0.1 ± 0.7	0.1 ± 0.9
Length (cm)	35.3 ± 3.1	35.2 ± 2.2	35.6 ± 3.0	34.9 ± 2.2
Length SDS	0.2 ± 1.1^{s}	-0.7 ± 1.0^{s}	-0.2 ± 1.0	-0.3 ± 1.3
Head circumference (cm)	25.1 ± 2.0	25.8 ± 1.4	25.5 ± 1.9	25.4 ± 1.5
Head circumference SDS	0.7 ± 1.2^{ss}	-0.1 ± 0.8^{ss}	0.2 ± 0.9	0.4 ± 1.3
At 2 years corrected age				
Weight (kg)	11.0 ± 1.2*	12.1 ± 1.0*	11.5 ± 1.2	11.6 ± 1.2
Weight SDS	-1.1 ± 1.0	-0,7 ± 0.7	-0.9 ± 0.9	-0.8 ± 0.8
Height (cm)	85.1 ± 3.4	87.2 ± 3.5	86.4 ± 4.3	85.9 ± 2.6
Height SDS	-0.8 ± 1.1	-0.5 ± 1.1	-0.6 ± 1.3	-0.7 ± 0.8
Total body fat (kg)	1.9 ± 0.7	1.7 ± 0.5	1.9 ± 0.6	1.8 ± 0.6
BMI (kg/m2)	15.2 ± 1.7	15.9 ± 1.1	15.5 ± 1.6	15.7 ± 1.3
BMI SDS	-0.8 ± 1.4	-0.4 ± 0.9	-0.7 ± 1.3	-0.5 ± 1.0
Systolic BP (mmHg)	92 ± 12	99 ± 14	92 ± 10	100 ± 16
Diastolic BP (mmHg)	58 ± 7	63 ± 8	59 ± 7	63 ± 9
Glucose (mmol/l)	4.4 ± 0.5	4.6 ± 0.5	4.6 ± 0.5	4.4 ± 0.4
Total cholesterol (mmol/l)	4.3 ± 0.8**	3.6 ± 0.6**	4.0 ± 0.8	3.8 ± 0.7
HDL cholesterol (mmol/l)	1.2 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.2 ± 0.3
Triglycerides (mmol/l)	1.2 ± 0.8	0.9 ± 0.4	1.3 ± 0.8***	0.7 ± 0.2***

All data are expressed as mean ± standard deviation. The total group of VLBW children (n = 38) is divided in boys and girls and divided in treatment group (standard care and early-insulin therapy). Standard deviation scores (SDS) at birth according to Niklasson et al. (29) and at 2 years corrected age according to Dutch references (20, 21). Total body fat was calculated according to Dauncey et al. (19) from skinfold thickness measurements and body dimensions.
Marked data are significantly different:
At birth:
- ^{s} length SDS boys vs. girls p = 0.008 (t-test)
- ^{ss} head circumference SDS boys vs. girls p = 0.013 (t-test)
At 2 years corrected age:
- * weight boys vs. girls p = 0.003 (t-test)
- ** total cholesterol boys vs. girls p = 0.006 (t-test)
- *** triglycerides early-insulin vs. standard care p = 0.003 (t-test)

Table 1 shows the characteristics at birth and 2 years corrected age with the group divided in male and female children and divided in early-insulin therapy and standard care. At birth, boys had a significant lower length SDS and head circumference SDS than girls. As expected, at 2 years corrected age, body weight was significantly higher in boys than in girls, but body weight SDS was not significantly different. Total cholesterol was significantly higher in girls than in boys. Triglycerides were significantly lower in children treated with insulin compared to children in the standard care group.

The most common component of the metabolic syndrome was systolic and/or diastolic blood pressure ≥ 90th percentile; this was present in 20 of the 32 children (63%) with known blood pressure. Twelve children (32%) had HDL cholesterol ≤ 1.03 mmol/l and 11 children (30%) had triglycerides ≥ 0.98 mmol/l. None of the children had BMI SDS > 2 or fasting glucose ≥ 5.6 mmol/l. Two children (5%) had three components of the metabolic syndrome, 13 children (34%) had two components and 11 children (29%) had one component. In 12 children (32%) there were no criteria of the metabolic syndrome, including 5 children with unknown blood pressure and 1 child with unknown lipids due to a small blood sample volume.

Table 2 shows the presence of the criteria of the metabolic syndrome with the group divided in male and female children and divided in early-insulin therapy and standard care. The prevalence of diastolic blood pressure ≥ 90th percentile was significantly higher among boys than girls. Children in the early-insulin group had a lower prevalence of triglycerides ≥ 0.98 mmol/l than children in the standard care group.

We did not find any significant correlations between body size and composition (weight, height, BMI, total body fat calculated according to Dauncey et al. (19)) and blood pressure, fasting glucose, HDL cholesterol and triglyceride levels. Table 3 shows HDL cholesterol for BMI SDS < 0 and BMI SDS > 0 in boys and girls and in the standard care and early-insulin group. Children with BMI SDS > 0 had significantly lower HDL cholesterol than children with BMI SDS < 0. In the group with BMI SDS > 0, boys had significantly lower HDL cholesterol than girls. In the group with BMI SDS < 0, children in the early-insulin group had significantly higher HDL cholesterol levels than children who received standard care. Blood pressure, fasting glucose and triglycerides were not significantly different between children with BMI SDS > 0 and BMI SDS < 0.

Diastolic blood pressure, fasting glucose, HDL cholesterol and triglycerides were not significantly correlated to parameters of growth (increments in weight (SDS), length (SDS) and head circumference (SDS)) between birth and term age, term and 6 months corrected age, 6 and 12 months corrected age and term and 2 years corrected age. Systolic blood pressure was significantly correlated with increment in length ($r = 0.39$; $p = 0.026$) and length SDS ($r = 0.42$; $p = 0.016$) between 6 and 12 months corrected age and with increment in weight ($r = 0.36$; $p = 0.047$), length ($r = 0.36$; $p = 0.046$) and length SDS ($r = 0.35$; $p = 0.048$) between term and 2 years corrected age.

Table 2. Presence of the metabolic syndrome components at the corrected age of 2 years

	Girls (n=19)	Boys (n=19)	Standard care (n=21)	Early-insulin (n=17)
BMI SDS > 2	0/19	0/19	0/21	0/17
Systolic BP ≥ p90	3/15 (20.0%)	4/17 (23.5%)	2/18 (11.1%)	5/14 (35.7%)
Diastolic BP ≥ p90	5/15 (33.3%)*	15/17 (88.2%)*	9/18 (50.0%)	11/14 (78.6%)
Systolic and/or diastolic BP ≥ p90	5/15 (33.3%)**	15/17 (88.2%)**	9/18 (50.0%)	11/14 (78.6%)
Glucose ≥ 5.6 mmol/l	0/19	0/19	0/21	0/17
HDL cholesterol ≤ 1.03 mmol/l	5/18 (27.8%)	7/19 (36.8%)	7/21 (33.3%)	5/16 (31.3%)
Triglycerides ≥ 0.98 mmol/l	6/18 (33.3%)	5/19 (26.3%)	10/21 (47.6%)***	1/16 (6.3%)***
1 metabolic syndrome component present	6/19 (31.6%)	5/19 (26.3%)	6/21 (28.6%)	5/17 (29.4%)
2 metabolic syndrome components present	5/19 (26.3%)	8/19 (42.1%)	7/21 (33.3%)	6/17 (35.3%)
3 metabolic syndrome components present	0/19	2/19 (10.5%)	2/21 (9.5%)	0/17

The total group of VLBW children (n = 38) is divided in boys and girls and divided in treatment group (standard care and early-insulin therapy). The data show the number of children in which the component is present, the total number of children in which the component is known and (between brackets) the percentage of children with the component present for all 4 subgroups.
Marked data are significantly different:
- * diastolic BP ≥ p90 boys vs. girls p = 0.001 (Chi-Square test)
- ** systolic and/or diastolic BP ≥ p90 boys vs. girls p = 0.001 (Chi-Square test)
- *** triglycerides ≥ 0.98 mmol/l early-insulin vs. standard care p = 0.01 (Fisher's Exact test)

Table 3. HDL cholesterol

		Total	Girls	Boys	Standard care	Early-insulin
BMI SDS < 0	**N**	25	13	12	16	9
	HDL	1.2 ± 0.3*	1.2 ± 0.3	1.3 ± 0.2**	1.2 ± 0.3^{s}	1.4 ± 0.1***^{s}
BMI SDS > 0	**N**	12	5	7	5	7
	HDL	1.0 ± 0.2*	1.1 ± 0.2^{n}	0.9 ± 0.1**^{n}	1.0 ± 0.2	1.0 ± 0.2***

All data are expressed as mean ± standard deviation. HDL cholesterol in mmol/l.
Marked data are significantly different:
- * BMI SDS < 0 vs. BMS SDS > 0 in total group p = 0.01 (t-test)
- ** BMI SDS < 0 vs. BMI SDS > 0 in boys p = 0.001 (t-test)
- *** BMI SDS < 0 vs. BMI SDS > 0 in early-insulin group p = 0.004 (t-test)
- ^{n} girls vs. boys in group with BMI SDS > 0 p = 0.045 (t-test)
- ^{s} early-insulin vs. standard care in group with BMI SDS < 0 p = 0.043 (t-test)

The only significant difference in growth between the early-insulin and standard care group was the change in weight SDS between 6 and 12 months corrected age (mean -0.09 vs. -0.50; p = 0.026). The mean increment in weight in this period was 2036 g in the early-insulin group (mean weight 7115 g (SDS -0.73) at 6 months and 9151 g (SDS -0.82) at 12 months) and 1707 g in the group with standard care (mean weight 7315 g (SDS -0.42) at 6 months and 9022 g (SDS -0.91) at 12 months) (p = 0.051). Weight, length

and head circumference at birth, term age, 6,12 and 24 months corrected age were not significantly different between the early-insulin and standard care group. There were no differences in growth between the children with and without any of the components of the metabolic syndrome.

DISCUSSION

Barker et al. (30) first indicated the importance of fetal growth for development of the metabolic syndrome and showed an increasing prevalence with decreasing birth weight. Insulin resistance plays a central role in the metabolic syndrome and adults born SGA are more insulin-resistant than controls (2, 3). Insulin sensitivity is already reduced in SGA born children, especially in children with catch-up growth (6, 7, 9, 11, 12).

Premature born infants have impaired growth in early postnatal life, in a period comparable to the third trimester of pregnancy. Adults born premature have higher fasting glucose levels, lower insulin sensitivity and higher blood pressure than controls (1, 4, 5). The reduction in insulin sensitivity as a result of premature birth is already present in children between the ages of 4 and 10 years (8). For VLBW infants, there is one study on insulin sensitivity in prepubertal children (age 5-7 years), which showed that insulin sensitivity is determined by growth in utero and postnatal growth rate (31). The other components of the metabolic syndrome have not been studied before in VLBW children.

The present study shows that the majority of VLBW infants already have one or more components of the metabolic syndrome at the corrected age of 2 years. Especially the prevalence of raised (diastolic) blood pressure is high. A possible explanation for the association between preterm birth and elevated blood pressure is reduced insulin sensitivity with hyperinsulinemia. Insulin levels are positively correlated to blood pressure in children (32); this could be mediated by the stimulating effect of insulin on sympathetic nervous system activity, renal sodium retention and/or vascular smooth muscle growth (33). Antenatal steroids could also contribute to the elevated blood pressure: almost all infants in our study received antenatal steroids and antenatal corticosteroid therapy is associated with higher systolic and diastolic blood pressures in later life (34). In our study the prevalence of raised diastolic blood pressure was significantly higher in boys than in girls. In adults the sex difference in blood pressure could possibly be explained by the differential effects of estrogens and androgens on the renin-angiotensin system (35). The hypothalamic-pituitary-gonadal axis is also active in the postnatal period and in girls follicle-stimulating hormone (FSH) levels and often also estradiol levels stay above those of older pre-pubertal children during the first years of life (36, 37). Therefore the blood pressure difference between boys and girls in our study could also be caused by sex hormones.

Almost one third of the VLBW children had high triglycerides. High childhood triglycerides are an important predictor of adult cardiovascular disease (38). We showed that the VLBW children treated with insulin in the first week of life had lower triglycerides than the children with standard care. By acting in a period that is critical for programming of insulin sensivity (8), early insulin therapy in VLBW infants could possibly improve long-term insulin sensitivity. The possible long-term benefit of early insulin treatment on triglyceride levels could then be the result of less insulin resistance as insulin resistance in the liver results in very low-density lipoprotein overproduction and development of hypertriglyceridemia (33, 39). As we did not measure insulin sensitivity in the present study, we could not confirm the role of insulin resistance in the development of high blood pressure and hypertriglyceridemia.

Low HDL cholesterol levels were also present in nearly one third of the children. We confirmed that a higher BMI is associated with an adverse metabolic profile, as children with BMI SDS > 0 had lower HDL cholesterol levels than children with BMI SDS < 0. The significant lower HDL cholesterol levels in boys with BMI SDS > 0 compared to girls with BMI SDS > 0 could be attributed to sex hormones, just like the significant higher total cholesterol levels in girls. Protein-bound estradiol is positively correlated with HDL cholesterol (40) and, as mentioned before, the hypothalamic-pituitary-gonadal axis is still active in 2-year-old girls. In the group with BMI SDS < 0, children treated with insulin had significantly higher HDL cholesterol levels than children who received standard care, suggesting that insulin treatment could also have advantages for this component of the metabolic syndrome. Longer follow-up is necessary to find out whether the possible metabolic advantages of early insulin treatment persist into later childhood and adulthood. Our proposal for longer follow-up is confirmed by previous results of animal studies in which leptin or exendin-4 was administered during the neonatal period, indicating that early postnatal intervention can indeed have long-term effects on metabolism (41, 42).

Rapid postnatal growth is unfavourable for blood pressure levels and insulin sensitivity of SGA and preterm born children and adults (4, 9-12, 15, 17, 31). In VLBW infants we demonstrated this already at the corrected age of 2 years: systolic blood pressure was positively correlated to parameters of growth between 6 and 12 months and between 0 and 24 months corrected age.

The results of our study have implications for the follow-up of VLBW infants. It is very important to measure blood pressure on a regular basis during childhood and some children, especially boys, will need treatment for hypertension. With the high prevalence of the components of the metabolic syndrome, parents should be counseled about the risk of cardiovascular disease and should be given life style advice.

Our study is limited by the small number of infants, the lack of data from term born children and the absence of a generally accepted definition of the metabolic syndrome

in children. The results have to be confirmed in a larger group of VLBW infants and compared with a control group of term born children. As the cut-off values for several metabolic syndrome components are now based on studies in other populations, the values of the VLBW children have to be compared with those of term born children from our own population.

In conclusion, this study is the first indication that at the corrected age of 2 years, VLBW infants already have a high prevalence of components of the metabolic syndrome. Raised (diastolic) blood pressure is most common, especially in boys. Early insulin treatment could possibly have long-term benefits for some components of the metabolic syndrome in VLBW infants. More studies are needed in larger groups of VLBW children, including control groups of term born children.

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Chapter 5

Components of the metabolic syndrome in early childhood in very-low-birth-weight infants and term small and appropriate for gestational age infants

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ABSTRACT

Background

Term small-for-gestational-age (SGA) and preterm born infants have an increased prevalence of metabolic syndrome components already in childhood. Our recent study in 2-year-old very-low-birth-weight (VLBW) infants was limited by the absence of a control group of term born children. We compared the metabolic syndrome components in early childhood in VLBW and term SGA infants to term appropriate-for-gestational-age (AGA) infants.

Methods

We included 38 VLBW children and 82 term born children (64 AGA/18 SGA). HDL cholesterol, triglycerides, glucose and insulin were measured in blood samples taken at 1 year (term children) and 2 years (all children) of (corrected) age.

Results

At 2 years corrected age, VLBW children have lower BMI and higher glucose level compared to AGA children. SGA children have lower BMI at 1 and 2 years of age and a high prevalence of high triglyceride levels at 1 year of age compared to AGA children. Total body fat is a significant determinant of HDL cholesterol and triglycerides and birth weight is a significant determinant of glucose at 2 years corrected age.

Conclusion

In early childhood, VLBW and term SGA children already have a high prevalence of some metabolic syndrome components compared to term AGA children.

INTRODUCTION

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. Besides life style and obesity, fetal and early postnatal growth are determinants of the metabolic syndrome. This is based on the studies of Barker et al. (1), who first indicated the importance of fetal growth for development of the metabolic syndrome in adulthood and showed an increasing prevalence with decreasing birth weight. Not only term small-for-gestational-age (SGA) born infants, but also preterm born infants have an increased prevalence of several components of the metabolic syndrome, already in childhood (2-9). The relation between metabolic syndrome components in childhood and fetal growth was studied by Jaddoe et al. (10); they showed that first trimester fetal growth restriction is associated with an adverse cardiovascular risk profile at 6 years of age.

We recently showed that at the corrected age of 2 years, very-low-birth-weight (VLBW) infants already have a high prevalence of components of the metabolic syndrome (11). In that study, 63% had raised blood pressure (systolic and/or diastolic ≥ 90th percentile for age, sex and height), 32% low HDL cholesterol (≤ 1.03 mmol/l) and 30% high triglycerides (≥ 0.98 mmol/l); none of the children had high BMI (BMI standard deviation score (SDS) > 2 for age and sex) or high glucose (≥ 5.6 mmol/l). This study was limited by the absence of a control group of term born children and consequently the cut-off values for several metabolic syndrome components were based on studies in other populations (12-15).

The aim of the present study was to compare the components of the metabolic syndrome in VLBW infants at the corrected age of 2 years with those in 2-year-old term appropriate-for-gestational-age (AGA) born children. The second aim was to compare the components of the metabolic syndrome in term SGA infants with those in term AGA infants at 1 and 2 years of age.

METHODS

Study population

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 (16). 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 included maternal diabetes and major congenital

anomalies. All infants participating in the NIRTURE trial in our neonatal intensive care unit were eligible for the present study.

The term infants were born from a low-risk population of pregnant women included in the first trimester in a prospective longitudinal study (Trophoblast study), which aimed to investigate the use of circulating trophoblast for prenatal diagnosis of pregnancy-associated diseases such as preeclampsia (17). The term born infants were divided in AGA and SGA; SGA was defined as a birth weight below the 10th percentile (18). Approval from the ethics committee of the VU University Medical Center was obtained.

Data collection

At the (corrected) ages of 3 months, 1 year and 2 years, anthropometry according to Dauncey et al. (19) was performed by a trained research nurse. Body weight was measured using an electronic scale to the nearest 0.1 kg, standing height was measured to the nearest 0.1 cm and all lengths and circumferences were measured using a measuring tape to the nearest 0.1 cm. BMI was calculated. Total body fat was calculated according to Dauncey et al. (19) from skinfold thickness measurements and body dimensions. SDS of weight, height and BMI were calculated according to Dutch references (20, 21). In the VLBW infants a blood sample was taken at 2 years corrected age and in the term born infants blood samples were taken at 1 and 2 years of age, according to the specific study protocol. All blood samples were taken after a fasting period of at least 3 hours. Blood samples were used for measurement of total cholesterol, HDL cholesterol, triglycerides, glucose and insulin. Insulin resistance was estimated by the homeostatic model assessment (HOMA = (fasting insulin mU/l x fasting glucose mmol/l)/22.5) (22). Blood pressure was only measured in VLBW infants at 2 years corrected age.

Assays

Total cholesterol, HDL cholesterol and triglycerides were measured by enzymatic colorimetric assay (CHOD-PAP, HDL-C plus and GPO-PAP, respectively; Modular Analytics, Roche diagnostics, Mannheim, Germany). Inter-assay coefficient of variation is 1.9% at both 3.5 mmol/l and 7.1 mmol/l for total cholesterol, 2.9% at 1.0 mmol/l and 2.8% at 2.4 mmol/l for HDL cholesterol and 3.0% at 1.1 mmol/l and 2.3% at 1.9 mmol/l for triglycerides.

Glucose concentrations were measured by the hexokinase method (Modular Analytics, Roche diagnostics, Mannheim, Germany). Inter-assay coefficients of variation are 2.0% at 4.8 mmol/l and 1.8% at 19.8 mmol/l.

Insulin was measured by immunometric assay (Advia Centaur, Siemens Medical Solutions Diagnostics, Malvern, Pennsylvania, USA). Lower limit of quantitation is 10 pmol/l, intra-assay coefficients of variation are 4% at 20 pmol/l, 3% at 500 pmol/l and 4% at 1500 pmol/l and inter-assay coefficients of variation are 8% at 24 pmol/l and 7% at both 780 pmol/l and 3000 pmol/l.

Metabolic syndrome components

According to the definition of Cook et al. (13), based on the criteria of the National Cholesterol Education Program (NCEP), Adult Treatment Panel III for the metabolic syndrome in adults (23), the metabolic syndrome is diagnosed in adolescents in the presence of at least 3 of the following 5 criteria: waist circumference ≥ 90th percentile; blood pressure (systolic and/or diastolic) ≥ 90th percentile; triglycerides ≥ 1.24 mmol/l (110 mg/dl); HDL cholesterol ≤ 1.03 mmol/l (40 mg/dl); fasting glucose ≥ 6.1 mmol/l (110 mg/dl) (13). As the metabolic syndrome as an entity should not be diagnosed in children younger than 10 years of age (15), we evaluated the components of the metabolic syndrome separately in the 1- and 2-year-old children in our study. Because waist circumference measurements were not part of the routine anthropometry, we used the definition of obesity of Weiss et al. (24): BMI SDS > 2 for age and sex.

In our recent study in 2-year-old VLBW infants (11), the cut-off values for blood pressure (systolic and/or diastolic ≥ 90th percentile for age, sex and height), triglycerides (≥ 0.98 mmol/l), HDL cholesterol (≤ 1.03 mmol/l) and glucose (≥ 5.6 mmol/l) were based on studies in other populations (12-15). The cut-off values for lipids were based on the NCEP Report of the Expert Panel on Blood Cholesterol Levels in Children and Adolescents; however, the percentile values in this report are based on studies that were performed more than 30 years ago, included only a small number of children less than 4 years old and made no distinction between term and preterm born children or AGA and SGA born children (12). The cut-off value for glucose was the same as the value used for adults, according to the International Diabetes Federation definition of the metabolic syndrome in children and adolescents (15). In the present study, we compare the values of the metabolic syndrome components in VLBW infants and term SGA infants with those of term AGA infants.

Statistical analysis

Statistical analyses were performed using the Statistical Package of Social Sciences software for Microsoft Windows version 19 (SPSS Inc., Chicago, Illinois, USA). Differences between (sub)groups were evaluated using Student's t-test for normally distributed values, Mann-Whitney test for not normally distributed values and Chi-Square tests. Bivariate correlation analysis was performed to study the relation between components of the metabolic syndrome, birth weight and parameters of body size and composition. Multiple regression analysis was used to determine which of these parameters were significant determinants of metabolic syndrome components at 1 and 2 years of (corrected) age. For insulin levels below the limit of quantitation, a value of 1 pmol/l was used. P values < 0.05 were considered as significant.

RESULTS

In our unit, 47 VLBW infants participated in the NIRTURE trial. Five infants died and 2 children were lost to follow-up. At the corrected age of 2 years, 40 children visited our outpatient clinic. Two children were excluded because the parents refused blood sampling, 38 VLBW children were included in the present study. Three (8%) of these VLBW children were SGA. Seventeen infants (9 male/8 female) were assigned to the early-insulin group and 21 infants (10 male/11 female) received standard neonatal care. We included all VLBW children as one group in the analyses; comparison between the early-insulin treated group and standard care group has been described earlier (11).

Ninety term born infants were included in the follow-up part of the Trophoblast study: 72 AGA and 18 SGA. Eight children were lost to follow-up after the first visit at 3 months of age; 82 term born children (64 AGA and 18 SGA) were included in the present study. Of these 82 children, 6 AGA children and 1 SGA child were lost to follow-up at 2 years of age.

Table 1 shows the gestational age and birth weight (SDS) of the VLBW children and term AGA and SGA children. For the VLBW children, the weight (SDS) at term age is also shown. Their weight (SDS) at term age is significantly lower than the birth weight (SDS) of term AGA children ($p < 0.001$) and significantly higher than the birth weight (SDS) of the term SGA children ($p < 0.001$).

Table 1. Characteristics of the VLBW and term (AGA and SGA) children at birth and term age

	VLBW (n=38) (19M/19F)	**Term AGA (n=64) (35M/29F)**	**Term SGA (n=18) (8M/10F)**
At birth			
Gestational age (wk)	27.9 ± 1.3*	39.3 ± 1.2	38.9 ± 1.2
Weight (g)	1059 ± 226*	3529 ± 393	2463 ± 251†
Weight SDS	-0.03 ± 0.8	0.3 ± 0.7	-2.1 ± 0.6†
At term age			
Weight (g)	3162 ± 558		
Weight SDS	-1.2 ± 1.3		

All data are expressed as mean ± standard deviation. Standard deviation scores (SDS) at birth according to Niklasson et al. (25) and at term age according to Dutch references (20).
VLBW infants are compared to term AGA infants. Marked data are significantly different: *$p < 0.01$
Term SGA infants are compared to term AGA infants. Marked data are significantly different: †$p < 0.01$

Anthropometry

Table 2 shows the weight (SDS), length (SDS) and total body fat at 3 months, 1 year and 2 years of (corrected) age for the VLBW children, term AGA and term SGA children. The data show that during the first 2 years of life, VLBW children and term SGA children stay lighter than term AGA children. In the first year of life they are also shorter, but at 2 years

of (corrected) age the length of VLBW and term SGA children does not differ significantly from term AGA children. In contrast to term SGA children, VLBW children have lower body fat percentage compared to term AGA children at all ages.

We compared the growth of VLBW children and term SGA children with the growth of term AGA children by comparing the change in weight (SDS) and length (SDS) between 0 and 3 months, 3 months and 1 year, 1 and 2 years and 0 months and 1 year/2 years (corrected) age. When comparing VLBW children to term AGA children, most differences were found between 3 months and 1 year corrected age. In VLBW children, the increment in length (SDS) was more than in term AGA children, resulting in the disappearance of the difference in length between VLBW and term AGA children with increasing age (Table 2). The increment in weight in this period was less in VLBW children compared to term AGA children. In term SGA children, the increment in weight SDS between birth and 3 months, birth and 1 year and birth and 2 years was significantly greater than in term AGA children.

Metabolic syndrome components

Table 3 shows the components of the metabolic syndrome in term (AGA and SGA) children at 1 and 2 years of age and in the VLBW children at 2 years of corrected age. At 2 years corrected age, VLBW children have lower BMI (SDS) but higher glucose levels than term AGA children. At 1 and 2 years of age, term SGA children have lower BMI (SDS) than term AGA children.

In table 4, we used the 75th and 90th percentile values of the metabolic syndrome components (25th and 10th percentile for HDL cholesterol) of our control group of term AGA children as cut-off values for the metabolic syndrome components in VLBW and term SGA children. The table shows the percentage of VLBW children and term SGA children with a value at or above the 75th and 90th percentile (for HDL cholesterol, at or below the 25th and 10th percentile). At 1 year of age, the prevalence of triglycerides ≥ p75 was significantly higher in term SGA children compared to AGA children. At 2 years of age, VLBW children had a higher prevalence of glucose ≥ p75 and glucose ≥ p90 than term AGA children.

Correlation and multiple regression analysis

In the term born children (AGA and SGA), HDL cholesterol at 1 year of age was significantly correlated to birth weight ($r = 0.26$; $p = 0.025$), but not to body size and composition (weight, length, BMI, total body fat) at 1 year. Glucose level at 1 year of age was significantly correlated to weight ($r = 0.54$; $p = 0.03$) only in SGA children, but not in AGA children. Triglycerides at 1 year of age were not correlated to birth weight or body size and composition at 1 year. In multiple regression analysis, only birth weight as predictor of HDL cholesterol at 1 year was significant.

Table 2. Weight, length and total body fat of the VLBW and term (AGA and SGA) children at 3 months, 1 year and 2 years of (corrected) age

	VLBW (n=38) (19M/19F)	Term AGA (n=64) (35M/29F)	Term SGA (n=18) (8M/10F)
At 3 months (corrected) age			
Weight (kg)	5.6 ± 0.9*	6.2 ± 0.6	5.4 ± 0.7†
Weight SDS	-0.5 ± 1.3*	0.5 ± 0.7	-0.5 ± 0.8†
Length (cm)	58.2 ± 2.7*	61.4 ± 2.0	58.7 ± 3.2†
Length SDS	-1.1 ± 1.1*	0.4 ± 0.7	-0.5± 1.1†
Total body fat (kg)	1.0 ± 0.5*	1.3 ± 0.3	1.0 ± 0.3†
TBF/weight	0.18 ± 0.06**	0.20 ± 0.04	0.19 ± 0.05
At 1 year (corrected) age			
Weight (kg)	9.1 ± 1.1*	10.1 ± 1.0	9.2 ± 1.0†
Weight SDS	-0.9 ± 1.0*	0.1 ± 0.8	-0.7 ± 0.9†
Length (cm)	74.5 ± 2.8*	76.5 ± 2.4	74.5 ± 3.3‡
Length SDS	-0.6 ± 1.0*	0.1 ± 0.8	-0.5 ± 1.3‡
Total body fat (kg)	1.7 ± 0.6 *	2.3 ± 0.7	1.9 ± 0.6‡
TBF/weight	0.18 ± 0.06*	0.22 ± 0.05	0.21 ± 0.05
At 2 years (corrected) age			
Weight (kg)	11.6 ± 1.2*	13.0 ± 1.5	11.9 ± 1.2†
Weight SDS	-0.9 ± 0.9*	0.1 ± 0.9	-0.6 ± 0.8†
Length (cm)	86.2 ± 3.6	87.7 ± 3.3	86.1 ± 4.7
Length SDS	-0.6 ± 1.1	-0.3 ± 0.9	-0.6 ± 1.4
Total body fat (kg)	1.8 ± 0.6*	2.6 ± 1.0	2.2 ± 0.6‡
TBF/weight	0.16 ± 0.05*	0.20 ± 0.06	0.18 ± 0.04

All data are expressed as mean ± standard deviation. Standard deviation scores (SDS) according to Dutch references (20).
VLBW children are compared to term AGA children. Marked data are significantly different: *$p < 0.01$ **$p < 0.05$
Term SGA children are compared to term AGA children. Marked data are significantly different: †$p < 0.01$ ‡$p < 0.05$

In the total group of children (term and VLBW), HDL cholesterol and triglycerides at 2 years of (corrected) age were significantly correlated to total body fat ($r = -0.21$; $p = 0.033$ and $r = 0.20$; $p = 0.038$, respectively) and to total body fat/weight ($r = -0.22$; $p = 0.021$ and $r = 0.23$; $p = 0.019$, respectively). Glucose at 2 years of (corrected) age was significantly correlated to birth weight ($r = -0.37$; $p < 0.001$). Only in term born children, glucose at 2 years of age was significantly correlated to total body fat ($r = 0.29$; $p = 0.016$), weight ($r = 0.32$; $p = 0.005$) and length ($r = 0.30$; $p = 0.009$) at 2 years of age. The strongest correlation of glucose to weight and length at 2 years was found in the SGA children ($r = 0.69$; $p = 0.002$ and $r = 0.51$; $p = 0.036$, respectively). Multiple regression analysis showed that total body fat is a significant determinant of HDL cholesterol and triglycerides and birth weight is a significant predictor of glucose at 2 years corrected age.

Table 3. Components of the metabolic syndrome, insulin and HOMA in VLBW and term (AGA and SGA) children at 1 and 2 years of (corrected) age

At 1 year (corrected) age		**Term AGA (n=64)**	**Term SGA (n=18)**
BMI (kg/m2)		17.2 ± 1.3	16.5 ± 1.3‡
BMI SDS		0.1 ± 0.9	-0.5 ± 1.0‡
Glucose (mmol/l)		3.9 ± 0.8	4.2 ± 0.4
Total cholesterol (mmol/l)		3.9 ± 0.8	3.5 ± 0.7
HDL cholesterol (mmol/l)		1.0 ± 0.3	0.9 ± 0.3
Triglycerides (mmol/l)		1.4 ± 0.7	1.8 ± 1.0
Insulin (pmol/l)		15.7 (1.0-179.4)	19.9 (1.0-86.5)
HOMA		0.4 (0.01-5.2)	0.5 (0.02-2.7)
At 2 years (corrected) age	**VLBW (n=38)**	**Term AGA (n=58)**	**Term SGA (n=17)**
BMI (kg/m2)	15.6 ± 1.5*	16.9 ± 1.1	16.0 ± 0.8†
BMI SDS	-0.6 ± 1.2*	0.5 ± 0.8	-0.1± 0.6†
Glucose (mmol/l)	4.5 ± 0.5*	4.0 ± 0.7	4.0 ± 0.5
Total cholesterol (mmol/l)	3.9 ± 0.8	4.1 ± 0.7	3.9 ± 0.7
HDL cholesterol (mmol/l)	1.2 ± 0.3	1.1 ± 0.3	1.0 ± 0.3
Triglycerides (mmol/l)	1.0 ± 0.7	1.2 ± 0.6	1.2 ± 0.5
Insulin (pmol/l)	21.0 (1.0-190.9)	17.9 (1.0-181.1)	20.4 (1.0-95.2)
HOMA	0.6 (0.02-6.5)	0.5 (0.01-5.6)	0.5 (0.02-2.9)

Data are expressed as mean ± standard deviation for normally distributed values and as median (range) for not normally distributed values.
VLBW children are compared to term AGA children. Marked data are significantly different: *$p < 0.01$
Term SGA children are compared to term AGA children. Marked data are significantly different: †$p < 0.01$ ‡$p < 0.05$

Insulin and HOMA

The results of the insulin measurements and HOMA calculations are also shown in table 3. There were no significant differences between VLBW and term AGA children and between term SGA and term AGA children.

DISCUSSION

The present study shows that 2-year-old VLBW infants have significantly higher glucose levels than term born AGA children. At 1 year of age, term born SGA children have a high prevalence of high triglycerides compared to AGA children.

In our recent study, we showed that, when using cut-off values from the literature, 2-year-old VLBW infants have a high prevalence of raised blood pressure, high triglyc-

Table 4. Classification of the components of the metabolic syndrome in VLBW and term SGA children based on the percentile values of term AGA children

At 1 year (corrected) age	**Term AGA**		**Term SGA**	
BMI	p75	18.0	≥ p75	2/18 (11%)
	p90	18.8	≥ p90	0/18
Glucose (mmol/l)	p75	4.5	≥ p75	3/16 (19%)
	p90	4.8	≥ p90	2/16 (13%)
HDL cholesterol (mmol/l)	p25	0.8	≤ p25	5/16 (31%)
	p10	0.7	≤ p10	4/16 (25%)
Triglycerides (mmol/l)	p75	1.7	≥ p75	9/16 (56%)[a]
	p90	2.4	≥ p90	2/16 (13%)

At 2 years (corrected) age	**Term AGA**		**VLBW**		**Term SGA**	
BMI	p75	17.5	≥ p75	4/38 (11%)	≥ p75	0/17
	p90	18.6	≥ p90	1/38 (3%)	≥ p90	0/17
Glucose (mmol/l)	p75	4.4	≥ p75	23/38 (61%)[b]	≥ p75	3/17 (18%)
	p90	4.5	≥ p90	19/38 (50%)[c]	≥ p90	2/17 (12%)
HDL cholesterol (mmol/l)	p25	0.9	≤ p25	8/37 (22%)	≤ p25	4/17 (24%)
	p10	0.7	≤ p10	4/37 (11%)	≤ p10	3/17 (18%)
Triglycerides (mmol/l)	p75	1.4	≥ p75	8/37 (22%)	≥ p75	5/17 (29%
	p90	1.5	≥ p90	6/37 (16%)	≥ p90	4/17 (24%)

The second column shows 75th and 90th percentile values (25th and 10th for HDL cholesterol) based on the term AGA children at 1 and 2 years of age. The next columns show for each metabolic syndrome component the number of term SGA and VLBW children with a value at or above this 75th and 90th percentile value (for HDL cholesterol at or below the 25th and 10th percentile value); the number of children is followed by the total number of children in which the component is known and (between brackets) the percentage of children with a value at or above/below the percentile value.
For marked data, the observed percentage is significantly higher than expected based upon the distribution in term AGA children:
-[a]triglycerides ≥ p75 in 1-year-old term SGA children $p = 0.019$ (Chi-Square test)
-[b]glucose ≥ p75 in 2-year-old VLBW children $p = 0.001$ (Chi-Square test)
-[c]glucose ≥ p90 in 2-year-old VLBW children $p < 0.001$ (Chi-Square test)

erides and low HDL cholesterol (11). In the present study, we could not confirm the high prevalence of high triglycerides and low HDL cholesterol in VLBW children. Fasting glucose levels of VLBW children were significantly higher than glucose levels of term AGA children. This is probably caused by reduced insulin sensitivity in VLBW children. In this small group, this hypothesis could not be confirmed with the HOMA values. Hofman et al. (4) showed that premature born children have reduced insulin sensitivity between 4 and 10 years of age (measured with the use of Bergman's minimal model from paired insulin and glucose data during an intravenous glucose-tolerance test). Our results suggest that VLBW children already have reduced insulin sensitivity at 2 years corrected age; this has to be confirmed by measurement of insulin sensitivity, preferably

by hyperinsulinemic euglycemic clamp technique, although this is hardly feasible at this age. In our earlier study, we did not find a high prevalence of high glucose levels (11).

The differences between the present study and our earlier study are caused by the findings that the cut-off value based on term born AGA children from our own population is higher for triglycerides, lower for HDL cholesterol and lower for glucose than the cut-off values used in our earlier study. In our opinion, the results of the present study are more reliable, as we now compare lipids and glucose of VLBW children with a control group of children of the same age and from the same population. The earlier used values were until now the best availabe cut-off values, but for lipids these cut-off values are based on studies in mainly older children from other parts of the world and performed more than 3 decades ago (12, 13) and for glucose this cut-off value is the same as used in adults (15). Therefore, these values are less appropriate to use as cut-off values for our VLBW children.

Previous studies show that school-aged SGA born children have reduced insulin sensitivity and higher blood pressure than AGA born controls (2, 3, 6, 8, 9). Reduced insulin sensitivity in SGA born infants can already be demonstrated in early childhood (5, 7). In the present study, we showed that at 1 year of age term born SGA children have a high prevalence of high triglycerides, with the cut-off value based on term born AGA children from the same population. The high prevalence of high triglycerides is probably caused by reduced insulin sensitivity, as insulin resistance in the liver results in very low-density lipoprotein overproduction and development of hypertriglyceridemia (26, 27). In this small group, this hypothesis could not be confirmed with the HOMA values. Our results are in accordance with those of Soto et al. (7); they found a tendency to higher triglyceride levels in 1-year-old SGA children compared to AGA children. High childhood triglycerides are an important predictor of adult cardiovascular disease (28). However, we did not find a significantly high prevalence of high triglycerides in 2-year-old SGA children. This is in contradiction to the concept of tracking metabolic abnormalities from childhood to adulthood in this population at risk. Studies in larger groups of SGA children are needed to clarify this.

The results of our study have implications for the follow-up of term SGA children and VLBW children. As term SGA children as well as VLBW children have a higher prevalence of some metabolic syndrome components compared to term AGA children, parents should be counseled about the risk of cardiovascular disease and given life style advices.

The most important limitation of our study is the small number of children. The results have to be confirmed in larger groups of VLBW and SGA children. More accurate measurement of insulin sensitivity in these children, ideally using hyperinsulinemic euglycemic clamp technique although hardly feasible, could contribute to the understanding of the pathophysiology that leads to an adverse metabolic profile in VLBW and SGA children. Unfortunately, blood pressure measurements of term born children were

not available, so comparison of blood pressure of VLBW children to term born children from our own population still has to be done.

In conclusion, in early childhood both VLBW and term SGA children already have a high prevalence of some metabolic syndrome components compared to term AGA children. VLBW children have significantly higher glucose levels than term born AGA children at 2 years corrected age. SGA children have a high prevalence of high triglyceride levels at 1 year of age. More studies are needed in larger groups of VLBW and SGA children, including more accurate measurement of insulin sensitivity and comparison of blood pressure to term AGA children.

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Chapter 6

Salivary and serum cortisol and relation to blood pressure in infancy and early childhood in very-low-birth-weight infants

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ABSTRACT

Background

Programming of the hypothalamic-pituitary-adrenal (HPA) axis possibly explains the relation between intra-uterine growth restriction (IUGR) and/or preterm birth and elevated blood pressure in later life. Very-low-birth-weight infants (birth weight < 1500 g) have high prevalence of raised blood pressure, already in early childhood. We investigated cortisol levels, relation to blood pressure and reliability of salivary cortisol in infancy and early childhood in very-low-birth-weight infants.

Methods

We included 41 children, participating in the randomized controlled Neonatal Insulin Replacement Therapy in Europe (NIRTURE) trial. Serum and salivary samples for cortisol measurement (immunoassay) were taken simultaneously at 6 months and separately at 2 years corrected age. Blood pressure was measured at 2 years corrected age.

Results

Serum cortisol was significantly correlated to systolic and diastolic blood pressure in boys and in the early-insulin treated group. At 2 years corrected age, serum cortisol was significantly higher in the early-insulin group compared to the standard care group. At 6 months corrected age, salivary cortisol was significantly correlated to serum cortisol.

Conclusions

In very-low-birth-weight boys, the positive correlation between cortisol and blood pressure is present at 2 years corrected age. Early insulin therapy could affect programming of the HPA axis. Salivary cortisol mirrors serum levels at 6 months corrected age.

INTRODUCTION

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. (1, 2) and confirmed in many studies in children and adults, reviewed by Huxley et al. (3). Preterm born infants have impaired growth in early postnatal life, in a period comparable to the third trimester of pregnancy, and also have a higher blood pressure in later life (4).

One of the proposed mechanisms underlying the association between intra-uterine growth restriction (IUGR) and/or preterm birth and blood pressure is programming of the hypothalamic-pituitary-adrenal (HPA) axis. In adults, birth weight is inversely associated with cortisol levels and cortisol levels are positively correlated to blood pressure (5, 6). This was also shown in children between the ages of 4.9 and 15.5 years and born at a gestational age > 32 weeks (7). In preterm born young adult men, cortisol is also associated with high systolic blood pressure (8). There are no data about the association between cortisol and blood pressure in preterm born infants < 32 weeks in early childhood. We recently showed that at the corrected age of 2 years, very-low-birth-weight (VLBW) infants (birth weight < 1500 g) have a high prevalence of raised blood pressure (systolic and/or diastolic ≥ 90th percentile for age, sex and height) (9). Elevated blood pressure (compared to published reference standards) in early childhood in VLBW infants was also reported by Duncan et al. (10).

The aim of the present study was to measure cortisol levels in VLBW infants at 6 months and 2 years corrected age and correlate cortisol levels at 2 years corrected age to blood pressure. Our second aim was to investigate the reliability of salivary cortisol measurements in this population. As the subjects were part of the Neonatal Insulin Replacement Therapy in Europe (NIRTURE) trial, our third aim was to evaluate the effect of early insulin therapy on salivary and serum cortisol levels at 6 months and 2 years corrected age. The blood pressure values were presented earlier, as part of the evaluation of all the metabolic syndrome components in this population (9). However, the emphasis of the present study is on cortisol, programming of the HPA axis and cortisol measurement in saliva compared to serum.

METHODS

Study population

The subjects were part of the NIRTURE trial, an international multicenter randomized controlled trial investigating the role of early insulin therapy in VLBW infants (11). After written informed consent was obtained from the parents, VLBW infants younger than 24 hours of age and requiring intensive care were randomized to receive continuous intra-

venous infusion of insulin for the first 7 days of life or standard neonatal care with insulin treatment only in case of hyperglycemia. Exclusion criteria included maternal diabetes and major congenital anomalies. All 47 infants participating in the NIRTURE trial in our neonatal intensive care unit were eligible for the present study. Therefore the sample size of the present study was determined by the number of infants we included in the NIRTURE trial. After discharge, all 42 surviving infants were followed in the outpatient clinic with visits at 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.

Data collection

At every visit to the outpatient clinic, body weight was measured using an electronic scale to the nearest 0.1 kg, length and head circumference were measured to the nearest 0.1 cm and BMI was calculated. Standard deviation scores (SDS) of weight, length and BMI were calculated according to Dutch references (12, 13). At 2 years corrected age, blood pressure was measured in the calm state using an appropriately sized cuff and automated blood pressure measuring device (Dinamap, Critikon, Tampa, Florida, USA); the mean value of two measurements was used for analysis and measurements in noncalm state were excluded. At 6 months and 2 years corrected age, blood and salivary samples were taken for measurement of cortisol. We only took blood samples at two of the visits to the outpatient clinic to limit the burden of blood sampling for the children. Saliva was collected by suction using a saliva aspiration set. At 6 months corrected age, this was performed by the research nurse in the outpatient clinic early in the afternoon and just before the blood sample was taken. At 2 years corrected age, saliva was collected by the parents at home immediately after awakening in the morning; the blood sample was taken early in the afternoon. As the saliva sample at 2 years corrected age was the only sample taken in the morning, comparison of 2-year salivary cortisol to the other cortisol values, all taken in the afternoon, was not possible.

Assays

Serum cortisol was measured by competitive immunoassay (Advia Centaur, Siemens Medical Solutions Diagnostics, Malvern, Pennsylvania, USA). Lower limit of quantitation is 30 nmol/l, intra-assay coefficient of variation is 3% at 700 nmol/l and inter-assay coefficients of variation are 6% at both 150 nmol/l and 500 nmol/l and 8% at 1000 nmol/l.

Salivary cortisol was measured by automated immunoassay (Architect i2000, Abbott, North Chicago, Illinois, USA). This assay is described in detail by Heijboer et al. (14).

Statistical analysis

Statistical analyses were performed using the Statistical Package of Social Sciences software for Microsoft Windows version 19 (SPSS Inc., Chicago, Illinois, USA). Differences between subgroups were evaluated using Student's t-test for normally distributed values and Mann-Whitney test and Wilcoxon signed-rank test for not normally distributed values. Bivariate correlation analysis was performed to study the relations between birth weight, cortisol concentrations and blood pressure and between serum and salivary cortisol concentrations. P values < 0.05 were considered as significant.

RESULTS

In our neonatal intensive care unit, 47 VLBW infants participated in the NIRTURE trial. Five infants died. One child was excluded because parents refused blood and salivary sampling at 6 months corrected age; at 2 years corrected age this child was lost to follow-up. Forty-one children were included in the present study. Table 1 shows the characteristics and outcome of these children. Seventeen infants (9 male/8 female) were assigned to the early-insulin group and 24 infants (12 male/12 female) received standard neonatal care. In the standard care group, 6 infants were treated with insulin for 1 or 2 days because of hyperglycemia due to sepsis.

Table 1. Characteristics and outcome of the VLBW children (n = 41)

Gestational age (wk) (mean (range))	27.9 (25.4-30.1)
Birth weight (g) (mean (range))	1059 (670-1460)
Birth weight SDS (mean (range))[a]	-0.1 (-2.7-1.3)
SGA[b]	4 / 41 (10%)
Sex	21 M / 20 F
Racial group	26 Caucasian, 10 Black, 3 Moroccan, 2 Asian
Highest level of parental education[c]	3 low, 18 medium, 20 high
Antenatal steroids (betamethasone i.m.)	39 / 41 (13 one dose, 24 two doses, 2 three doses)
Postnatal steroids (hydrocortisone)	2 / 41
Bronchopulmonary dysplasia[d]	8 / 41 (20%)
Outcome at 2 years corrected age	3 developmentally delayed; 3 cerebral palsy

[a]Standard deviation scores (SDS) at birth according to Niklasson et al. (15).
[b]Small-for-gestational-age (SGA) was defined as a birth weight below the 10th percentile.
[c]Highest level of education completed by either parent was used as an indicator of socioeconomic status and classified as low (primary school, low occupational training), medium (high school, medium occupational training) or high (high occupational training, university).
[d]Bronchopulmonary dysplasia was defined as the need for supplemental oxygen at 36 weeks postmenstrual age.

Table 2 shows serum and salivary cortisol at 6 months and 2 years corrected age and blood pressure at 2 years corrected age with the group divided in male and female children and divided in early-insulin therapy and standard care. At 2 years corrected age, serum cortisol was significantly higher in children treated with insulin compared to children in the standard care group. Paired-samples t-test showed no differences between serum cortisol levels at 6 months and 2 years corrected age (both taken in the afternoon).

Table 2. Cortisol and blood pressure

	Girls (n=20)	**Boys (n=21)**	**Standard care (n=24) (12M/12F)**	**Early-insulin (n=17) (9M/8F)**
At 6 months corrected age				
Cortisol serum (nmol/l)[a]	288 (68-435)	208 (95-328)	210 (68-435)	260 (92-380)
Cortisol saliva (nmol/l)[a]	3.5 (0.1-20.3)	4.1 (0.1-9.1)	3.6 (0.1-6.6)	4.9 (0.1-20.3)
At 2 years corrected age				
Systolic BP (mmHg)	91 (75-120)	96 (82-133)	92 (75-115)	97 (81-133)
Diastolic BP (mmHg)	56 (51-79)	61 (50-81)	57 (50-73)	60 (51-81)
Cortisol serum (nmol/l)[a]	191 (67-671)	199 (80-828)	163 (67-573)[c]	272 (154-828)[c]
Cortisol saliva (nmol/l)[b]	11.7 (3.5-37.7)	9.7 (2.4-41.3)	11.5 (3.7-41.3)	6.8 (2.4-35.9)

All data are expressed as median and range. The total group of VLBW children (n = 41) is divided in boys and girls and divided in treatment group (standard care and early-insulin therapy). Marked data are significantly different.

[a] sample taken in the afternoon.

[b] sample taken in the morning.

[c] cortisol serum at 2 years corrected age: standard care vs. early-insulin p = 0.002 (Mann-Whitney test).

At 2 years corrected age, serum cortisol (taken at the visit to the outpatient clinic early in the afternoon) was significantly correlated to both systolic blood pressure and diastolic blood pressure in boys ($r = 0.79$; $p < 0.001$ and $r = 0.65$; $p = 0.004$ resp.), but not in girls ($r = 0.39$; $p = 0.15$ and $r = 0.47$; $p = 0.08$ resp.) and in the early-insulin group ($r = 0.80$; $p = 0.001$ and $r = 0.68$; $p = 0.008$ resp.), but not in the standard care group ($r = 0.33$; $p = 0.19$ and $r = 0.36$; $p = 0.14$ resp.). These correlations are shown in Figure 1. There was no correlation between salivary cortisol (taken at home early in the morning) and blood pressure. Birth weight was not correlated to serum or salivary cortisol at any age or to blood pressure at 2 years corrected age. At 6 months corrected age, salivary cortisol was significantly correlated to serum cortisol ($r = 0.62$; $p = 0.001$).

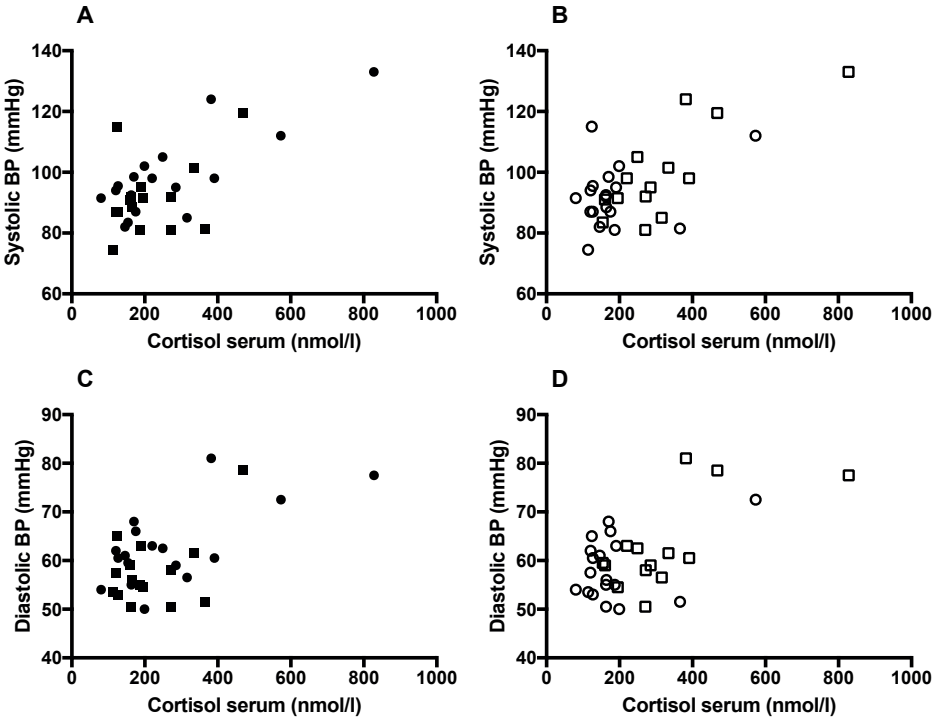

Figure 1. Correlation between blood pressure (BP) and serum cortisol at 2 years corrected age with the group of VLBW children divided in boys (●) and girls (■) (A for systolic BP en C for diastolic BP) and divided in treatment group (standard care (○) and early-insulin therapy (□)) (B for systolic BP and D for diastolic BP).

DISCUSSION

The present study shows that in subgroups of VLBW infants, the positive correlation between cortisol and blood pressure can be demonstrated as early as at 2 years corrected age. At 2 years corrected age, insulin-treated children have higher serum cortisol levels than children in the standard care group. Salivary cortisol measurement is reliable in 6-month-old VLBW children.

The positive correlation between serum cortisol levels and blood pressure has been shown before in adults and older children (5-7). The present study confirms this association in early childhood in VLBW infants and supports the hypothesis that programming of the HPA axis may contribute to the high prevalence of raised blood pressure in this population (9, 10). The correlation between cortisol and both systolic and diastolic blood pressure was significant only in boys. This is in accordance with the study of Szathmari et al. (8) in preterm born young adults, showing an association between cortisol and high systolic blood pressure only in men. In older children (between the ages of 4.9 and 15.5

years), including preterm born children > 32 weeks gestation, the association between cortisol levels and blood pressure was not different between boys and girls (7). The age groups, which show the sex difference in this association (adulthood and early childhood), correspond to periods that the hypothalamic-pituitary-gonadal axis is active, as this is also active in the postnatal period (16, 17).

VLBW children treated with insulin in the first postnatal week, have higher serum cortisol levels at 2 years corrected age than children treated with standard care. In the insulin treated group, there also was a significant correlation between serum cortisol levels and blood pressure (systolic and diastolic). These results suggest that early insulin treatment may affect the programming of the HPA axis, although the study population was small. Animal studies in offspring of diabetic mothers show that increased insulin concentrations within the immature hypothalamus may lead to irreversible malprogramming (with morphological changes) of regulation centres for metabolism and body weight (18).

Proposed mechanisms for the positive relation between increased HPA axis activity and blood pressure are reduced insulin sensitivity and activation of the central sympathetic nervous system (19). The absence of a significant correlation between salivary cortisol and blood pressure at 2 years corrected age could be caused by the small population of our study. On the other hand, the fact that only afternoon (serum) and not morning (salivary) cortisol levels were correlated to blood pressure, could also indicate that the failure to suppress cortisol during the day, resulting in sustained hypercortisolism, is related to elevated blood pressure.

In this study, we found a significant correlation between salivary and serum cortisol levels in VLBW infants at 6 months corrected age. This finding confirms the reliability of salivary cortisol measurements shown before in premature infants (20) and older children (21). We did not find the inverse relation between birth weight and cortisol levels as shown in other studies in children (7, 22).

Our study is limited by the small number of infants and the lack of data from term born children. The correlations between cortisol and blood pressure need to be confirmed in a larger group of VLBW infants. Cortisol levels and blood pressure have to be compared with those of term born children from our own population.

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 could affect this programming, resulting in higher cortisol levels. Salivary cortisol mirrors serum levels at 6 months corrected age and has an important advantage as non-invasive method, especially in children.

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Chapter 7

Cortisol and cortisone in early childhood in very-low-birth-weight infants and term born infants

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Submitted

ABSTRACT

Objective

Besides programming of the hypothalamic-pituitary-adrenal (HPA) axis, changes in the activity of 11ß-hydroxysteroid dehydrogenase type 2 (11ß-HSD2) could contribute to the later metabolic and cardiovascular consequences of preterm birth. We compared serum cortisol, cortisone and cortisol/cortisone ratio and its relationships to metabolic syndrome components in early childhood in very-low-birth-weight infants and term appropriate-for-gestational-age born infants.

Design

Observational study.

Patients

We included 41 very-low-birth-weight infants, participating in the randomized controlled Neonatal Insulin Replacement Therapy in Europe trial, and 64 term appropriate-for-gestational-age born infants.

Measurements

Cortisol and cortisone were measured in blood samples taken at 6 months and 2 years corrected age (very-low-birth-weight children) and at 3 months, 1 and 2 years (term children). At 1 year and 2 years of (corrected) age (HDL) cholesterol, triglycerides, glucose and insulin were also measured.

Results

During the first 2 years of life, cortisol/cortisone ratio is higher in very-low-birth-weight children compared to term children. Cortisol/cortisone ratio is related to several metabolic syndrome components in term children and in subgroups of very-low-birth-weight children with high glucose or low HDL cholesterol levels. In very-low-birth-weight children, over the first 2 years of life both cortisol and cortisone are higher in the early-insulin group compared to the standard care group.

Conclusions

In very-low-birth-weight infants, lower 11ß-HSD2 activity probably contributes to the long-term metabolic and cardiovascular risks. In very-low-birth-weight infants, early insulin treatment could affect programming of the HPA axis, resulting in higher cortisol and cortisone levels during early childhood.

INTRODUCTION

Programming of the hypothalamic-pituitary-adrenal (HPA) axis plays an important role in the association between intra-uterine growth restriction (IUGR) and/or preterm birth and higher blood pressure in later life. Very-low-birth-weight (VLBW) infants have a high prevalence of raised blood pressure already in early childhood (1-3) and we earlier showed that at the corrected age of 2 years, cortisol levels are positively correlated to blood pressure in VLBW boys (4).

Studies in IUGR born children suggest that changes in the activity of 11ß-hydroxysteroid dehydrogenase type 2 (11ß-HSD2) contribute to the metabolic and cardiovascular consequences in later life (5, 6). 11ß-HSD2 converts cortisol into inactive cortisone and is mainly active in the kidney (7). In children born with IUGR and without catch-up growth, cortisol (F)/cortisone (E) ratio at the mean age of 7 years was significantly higher compared to controls, suggesting a partial 11ß-HSD2 deficit (5). In these children, F/E ratio was positively correlated with cholesterol levels, indicating a risk factor for cardiovascular disease (5). We hypothesize that changes in 11ß-HSD2 activity could also contribute to the later consequences of preterm birth.

The aim of the present study was to compare serum cortisol, cortisone and F/E ratio in infancy and early childhood in VLBW infants (birth weight < 1500 g) to term appropriate-for-gestational-age (AGA) born infants. The VLBW children were part of the Neonatal Insulin Replacement Therapy in Europe (NIRTURE) trial (8). We showed earlier that at 2 years corrected age, insulin-treated children had higher serum cortisol levels than children in the standard care group (4). In the present study, we also evaluate the effect of early insulin therapy on serum cortisone and F/E ratio. The third aim was to investigate the relationships between F/E ratio and components of the metabolic syndrome in (subgroups of) VLBW children and term born AGA children. The cortisol levels of the VLBW children were presented earlier, in relation to blood pressure (4). The present study focuses on the F/E ratio and the comparison to term born AGA children.

METHODS

Study population

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 (8). 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 included maternal diabetes and major congenital anomalies. All infants participating in the NIRTURE trial in our neonatal intensive care unit (inclusion period from 2006 to 2007) were eligible for the present study. Therefore the sample size of the VLBW infants in the present study was determined by the number of infants we included in the NIRTURE trial. The results of the NIRTURE trial did not show short-term clinical benefits of early insulin therapy (8); long-term results have not yet been published.

The term infants were born between 2000 and 2005 from a low-risk population of pregnant women included in the first trimester in a prospective longitudinal study (Trophoblast study), which aimed to investigate the use of circulating trophoblast for prenatal diagnosis of pregnancy-associated diseases such as preeclampsia (9). Only term infants born AGA were included in the present study. AGA was defined as a birth weight above the 10th percentile (10). Standard deviation scores (SDS) of birth weight were calculated according to Niklasson et al. (11). Approval from the ethics committee of the VU University Medical Center was obtained.

During the inclusion period of the NIRTURE trial in our neonatal intensive care unit (21 months), 165 VLBW infants were admitted and the parents of 69 infants were approached regarding participation in the study. The most common reasons for not approaching parents were infants not requiring intensive care or no opportunity to obtain informed consent within the first 24 hours after birth. In our unit, 47 VLBW infants participated in the NIRTURE trial. Five infants died and one child was excluded because parents refused blood sampling at the follow-up visits; 41 VLBW children were included in the present study. At 2 years corrected age, one of these 41 children was lost to follow-up. Four (10%) of the VLBW children were SGA (defined as a birth weight below the 10th percentile (10)). Thirty-nine infants received antenatal steroids (one (n = 13), two (n = 24) or three (n = 2) doses of betamethasone intramuscularly) and 2 received postnatal steroids (hydrocortisone). Seventeen infants (9 male/8 female) were assigned to the early-insulin group and 24 infants (12 male/12 female) received standard neonatal care. During the first week of life, 6 infants in the standard care group were treated with insulin for 1 or 2 days because of hyperglycemia due to sepsis.

Ninety term born infants were included in the follow-up part of the Trophoblast study of whom 72 were AGA. Eight AGA children were excluded from the present study because they were lost to follow-up after the first visit at 3 months of age; 64 children were included in the present study. At 2 years of age, 6 children were lost to follow-up.

Data collection

The VLBW infants visited the outpatient clinic at expected date of delivery and at the corrected ages of 3 and 6 months, 1 year and 2 years, the term born infants at 3 months, 1 year and 2 years of age, according to the protocol of the NIRTURE trial and Trophoblast

study, respectively. At each visit, anthropometry according to Dauncey et al. (12) was performed by the same trained research nurse in all children. Standard deviation scores of weight, height, head circumference and BMI were calculated according to Dutch references (13, 14). Blood samples for measurement of cortisol and cortisone were taken at 6 months and 2 years corrected age in the VLBW infants and at 3 months, 1 and 2 years of age in the term born infants, according to the specific study protocol. Blood samples taken at 1 year and 2 years of (corrected) age were also used for measurement of total cholesterol, HDL cholesterol, triglycerides, glucose and insulin. Insulin resistance was estimated by the homeostatic model assessment (HOMA = (fasting insulin mU/l x fasting glucose mmol/l)/22.5) (15). All blood samples were taken early in the afternoon after a fasting period of at least 3 hours. Samples were stored at -80 ^{o}C and were all analysed at the same time. Study population and data collection also have been previously described (1, 4, 16, 17).

Assays

For measurement of serum cortisol and cortisone, samples were prepared as described by Hawley et al. (18); concentrations were assessed by isotope dilution LC-tandem MS method, as described in detail by van der Voorn et al. (19, 20).

Total cholesterol, HDL cholesterol and triglycerides were measured by enzymatic colorimetric assay (CHOD-PAP, HDL-C plus and GPO-PAP, respectively; Modular Analytics, Roche diagnostics, Mannheim, Germany). Inter-assay coefficient of variation is 1.9% at both 3.5 mmol/l and 7.1 mmol/l for total cholesterol, 2.9% at 1.0 mmol/l and 2.8% at 2.4 mmol/l for HDL cholesterol and 3.0% at 1.1 mmol/l and 2.3% at 1.9 mmol/l for triglycerides.

Glucose concentrations were measured by the hexokinase method (Modular Analytics, Roche diagnostics, Mannheim, Germany). Inter-assay coefficients of variation are 2.0% at 4.8 mmol/l and 1.8% at 19.8 mmol/l.

Insulin was measured by immunometric assay (Advia Centaur, Siemens Medical Solutions Diagnostics, Malvern, Pennsylvania, USA). Lower limit of quantitation is 10 pmol/l, intra-assay coefficients of variation are 4% at 20 pmol/l, 3% at 500 pmol/l and 4% at 1500 pmol/l and inter-assay coefficients of variation are 8% at 24 pmol/l and 7% at both 780 pmol/l and 3000 pmol/l. For insulin levels below the limit of quantitation a value of 1 pmol/l was used.

Statistical analysis

Statistical analyses were performed using the Statistical Package of Social Sciences software for Microsoft Windows version 19 (SPSS Inc., Chicago, Illinois, USA) and Stata version 14 (StataCorp, College Station, Texas, USA). Differences in characteristics between VLBW children and term AGA children were evaluated using independent Student's t-test for

normally distributed variables, Mann-Whitney test for not normally distributed variables and chi-square tests for dichotomous and categorical variables. Multiple regression analysis was used to assess the effect of antenatal steroids on cortisol, cortisone and F/E ratio in VLBW children.

Longitudinal differences in serum cortisol, cortisone and F/E ratio between the VLBW children and term AGA children and between the subgroups of VLBW children (boys versus girls and early-insulin versus standard care) were analysed with linear mixed model analyses. The longitudinal relationship between the F/E ratio and several components of the metabolic syndrome was also investigated with linear mixed model analyses. Linear mixed model analyses were used to adjust for the dependency of the observations within one child. Both cortisol and cortisone were log transformed before analyses and all analyses were (if possible) adjusted for gender. P values < 0.05 were considered as significant.

In addition, we investigated the relationships between F/E ratio and metabolic syndrome components in subgroups of VLBW children, in which metabolic risk factors are already present at 2 years corrected age. These subgroups were defined using the cut-off values for glucose, HDL cholesterol and triglycerides based on the term AGA children as we described earlier (16). The relationships were analyzed with linear regression analyses adjusted for gender.

RESULTS

Table 1 shows the characteristics of the VLBW infants and term AGA infants including parental background information. Anthropometric data were reported earlier (17).

Cortisol and cortisone

Table 2 shows serum cortisol, serum cortisone and F/E ratio for the VLBW and term AGA children. At 2 years (corrected) age, F/E ratio was significantly higher in VLBW children compared to term AGA children ($p = 0.039$). Longitudinal analysis showed that on average over the first 2 years of life, F/E ratio was significantly higher in VLBW children compared to term AGA children. Serum cortisol and cortisone did not differ significantly between the VLBW and term AGA children over time.

In VLBW children, cortisone at 2 years corrected age was significantly lower than at 6 months corrected age ($p = 0.008$) and F/E ratio was significantly higher at 2 years compared to 6 months corrected age ($p < 0.001$). In term AGA children, cortisone at 1 year of age was significantly lower than at 3 months of age ($p < 0.001$) and F/E ratio was significantly higher at 1 year compared to 3 months of age ($p < 0.001$).

Table 1. Characteristics of the VLBW and term AGA children

	VLBW (n=41)	Term AGA (n=64)	p-value
Sex	21 M / 20 F	35 M / 29 F	0.73
Gestational age (wk)	27.9 ± 1.3	39.3 ± 1.2	< 0.001
Birth weight (g)	1059 ± 231	3529 ± 393	< 0.001
Birth weight SDS	-0.06 ± 0.9	0.3 ± 0.7	0.02
Maternal age (years)	31.3 ± 4.7	33.7 ± 4.4	0.01
Maternal weight (kg)	68.2 ± 13.7	71.7 ±13.2	0.21
Maternal smoking	5/41 (12%)	6/64 (9%)	0.75
Racial group	26 Caucasian, 10 Black, 3 Moroccan, 2 Asian	55 Caucasian, 4 Black, 5 Asian	
Highest level of parental education[a]	3 low, 18 medium, 20 high	1 low, 19 medium, 35 high, 9 unknown	
Breast feeding	31/41 (76%)	45/64 (70%)	0.55
- duration of exclusive breast feeding (months)	3 (0-8)	3 (0-6)	0.21
- total duration of breast feeding (months)	5 (1-23)	4 (1-24)	0.92
Weight (g) at expected date of delivery[b]	3154 ± 579		< 0.001
Weight SDS at expected date of delivery[b]	-1.2 ± 1.3		< 0.001

Data are expressed as mean ± standard deviation, percentages or numbers; duration of breast feeding is presented as median (range).
VLBW infants are compared to term AGA infants.
[a]Highest level of education completed by either parent was used as an indicator of socioeconomic status and classified as low (primary school, low occupational training), medium (high school, medium occupational training) or high (high occupational training, university).
[b]Weight (SDS) at expected date of delivery of the VLBW infants was compared to birth weight (SDS) of the term AGA infants.

Table 3 shows serum cortisol and cortisone in subgroups of VLBW children. Longitudinal analysis showed that on average over the first 2 years of life, both cortisol and cortisone were significantly higher in the early-insulin group compared to the standard care group. F/E ratio did not differ between these 2 groups. We did not find significant differences between VLBW boys and girls. Adjustment for the number of doses of antenatal steroids did not influence these results.

Relation of F/E ratio to metabolic syndrome components

Table 4 shows the longitudinal relationship over the first 2 years of life between the F/E ratio and the metabolic syndrome components in the VLBW children and the term AGA children. Descriptive information regarding these measurements was reported earlier (16). In term AGA children, there was a significant relationship between F/E ratio and total cholesterol, glucose, insulin and HOMA over the first 2 years of life. For the VLBW children no significant relationships were found.

Table 2. Serum cortisol, cortisone and ratio

	VLBW (n=41)	Term AGA (n=64)
At 3 months (corrected) age		
Cortisol (nmol/l)		255 (27-750)
Cortisone (nmol/l)		89 (31-176)
Ratio cortisol/cortisone		2.8 (0.8-8.6)
At 6 months (corrected) age		
Cortisol (nmol/l)	215 (62-434)	
Cortisone (nmol/l)	65 (21-94)	
Ratio cortisol/cortisone	3.7 (1.5-5.9)	
At 1 year (corrected) age		
Cortisol (nmol/l)		245 (88-693)
Cortisone (nmol/l)		57.5 (20-92)
Ratio cortisol/cortisone		4.3 (2.5-10.5)
At 2 years (corrected) age		
Cortisol (nmol/l)	200 (75-824)	243 (54-729)
Cortisone (nmol/l)	47 (19-101)	61 (12-89)
Ratio cortisol/cortisone	4.7 (2.3-11.2)	4.1 (2.8-9.7)
Difference between the VLBW and term AGA children on average over time adjusted for gender		
Cortisol (nmol/l)	0.99[a] (0.79 to 1.22); p = 0.89	
Cortisone (nmol/l)	0.88[a] (0.75 to 1.03); p = 0.10	
Ratio cortisol/cortisone	0.62 (0.06 to 1.19); p = 0.03	

Data are expressed as median and range.
[a]Difference expressed as a ratio (VLBW compared to term AGA; VLBW lower values than term AGA).

Additional analyses

In 2-year-old VLBW children with high glucose levels (≥ p75; n = 23), F/E ratio was significantly related to triglycerides (standardized regression coefficient = 0.52; p = 0.03); there were no significant relationships between F/E ratio and HDL cholesterol, glucose and insulin. In VLBW children with low HDL cholesterol (≤ p25; n = 8), F/E ratio was significantly related to glucose levels (standardized regression coefficient = 0.78; p = 0.04); there were no significant relationships between F/E ratio and HDL cholesterol, triglycerides and insulin. In VLBW children with high triglycerides, we did not find significant relationships between F/E ratio and any of the metabolic syndrome components.

Table 3. Serum cortisol, cortisone and ratio in subgroups of VLBW children

	Girls (n=20)	Boys (n=21)	Standard care (n=24)	Early-insulin (n=17)
At 6 months corrected age				
Cortisol (nmol/l)	233 (62-434)	200.5 (99-389)	203.5 (62-434)	242 (92-407)
Cortisone (nmol/l)	70.5 (21-94)	63.5 (30-82)	63 (21-92)	68.5 (29-94)
Ratio cortisol/cortisone	3.8 (2.0-5.9)	3.7 (1.5-5.4)	3.7 (1.5-5.9)	3.8 (2.2-5.4)
At 2 years corrected age				
Cortisol (nmol/l)	207.5 (75-648)	195 (79-824)	181 (75-526)	312.5 (179-824)
Cortisone (nmol/l)	44 (25-91)	47 (19-101)	36 (19-89)	72 (33-101)
Ratio cortisol/cortisone	4.7 (3.0-7.1)	4.8 (2.3-11.2)	4.6 (3.0-11.2)	5.2 (2.3-8.2)
Difference between the groups on average over time				
Cortisol (nmol/l)	0.99[a] (0.78 to 1.36); p = 0.93		1.39[a] (1.12 to 1.75); p = 0.003	
Cortisone (nmol/l)	0.99[a] (0.82 to 1.19); p = 0.90		1.28[a] (1.08 to 1.53); p = 0.005	
Ratio cortisol/cortisone	0.09 (-0.59 to 0.72); p = 0.80		0.31 (-0.38 to 0.99); p = 0.38	

Data are expressed as median and range.
[a]Difference expressed as a ratio (boys versus girls and early-insulin versus standard care).

Table 4. Longitudinal relationship between cortisol/cortisone ratio and metabolic syndrome components

	VLBW	Term AGA
Total cholesterol (mmol/l)	-0.06 (-0.20 to 0.08); p = 0.39[a]	-0.10 (-0.18 to -0.02); p = 0.01
HDL cholesterol (mmol/l)	-0.01 (-0.07 to 0.06); p = 0.82[a]	-0.02 (-0.05 to 0.01); p = 0.22
Triglycerides (mmol/l)[b]	0.08 (-0.02 to 0.18); p = 0.10[a]	-0.003 (-0.06 to 0.05); p = 0.92
Glucose (mmol/l)	0.01 (-0.10 to 0.11); p = 0.87[a]	-0.10 (-0.20 to -0.01); p = 0.04
Insulin (pmol/l)[b]	0.04 (-0.14 to 0.22); p = 0.68	-0.24 (-0.42 to -0.06); p = 0.01
HOMA[b]	-0.09 (-0.37 to 0.20); p = 0.55[a]	-0.27 (-0.47 to -0.07); p = 0.01

All analyses were adjusted for gender. For example, in term AGA children a difference of 1 unit in cortisol/cortisone ratio is associated with a difference of -0.10 units in total cholesterol on average over time.
[a]Relationship could only be assessed on the measurements at 2 years corrected age.
[b]Analyses were performed on log transformed values.

DISCUSSION

The present study shows that during the first 2 years of life, F/E ratio is significantly higher in VLBW children compared to term AGA children. Insulin-treated VLBW children have higher serum cortisol and cortisone levels during the first 2 years of life than VLBW children in the standard care group. In early childhood, F/E ratio is related to several metabolic syndrome components.

In healthy subjects, F/E ratio rises during the first year of life, stays unchanged until adulthood and then increases with age (21). The increase of F/E ratio indicates a decrease in 11ß-HSD2 activity and may contribute to the increase of blood pressure in early childhood and in elderly persons (22). This is in accordance with the results of the present study, we also found an increase in F/E ratio in early childhood, in both VLBW children and term AGA children.

Early programming of the HPA axis, resulting in increased activity in later life, probably plays an important role in the later metabolic and cardiovascular consequences of preterm birth. In childhood, preterm born infants have reduced insulin sensitivity and raised blood pressure (23-25). Several studies show higher salivary cortisol levels in preterm born children aged 5-14 years compared to term born controls (26-28) and cortisol levels are positively correlated to blood pressure in children (29), indicating the role of programming of the HPA axis.

Some of the components of the metabolic syndrome can already be detected at preschool age in preterm born children. We showed earlier that at 2 years corrected age, VLBW children have significantly higher glucose levels than 2-year-old term born AGA children (16). Blood pressure is already elevated in early childhood in VLBW infants (1-3). The positive correlation between serum cortisol and blood pressure we showed earlier in VLBW boys at 2 years corrected age, suggests the contribution of programming of the HPA axis already in early childhood (4). In accordance with this, Grunau et al. (30) showed that preterm infants born at less than 28 weeks of gestational age, had significantly higher basal salivary cortisol levels at 8 and 18 months corrected age compared to term born infants.

In the present study, serum cortisol levels were not higher in VLBW infants, but over the first 2 years of life F/E ratio was higher in VLBW children compared to term AGA children. As far as we know, F/E ratio has not earlier been investigated in early childhood in VLBW infants. Houang et al. (5) studied F/E ratio in IUGR born children without catch-up growth at 1.1 to 13.5 years of age. They showed that 20% of these children had high F/E ratios compared to controls, suggesting partial 11ß-HSD2 deficit, and that high F/E ratio was associated with high cholesterol levels and high blood pressure (5). In another study in SGA born children at the age of 12 years, high F/E ratio was associated with high LDL cholesterol and high HOMA (6). Considering these associations with cholesterol, blood pressure and insulin resistance, high F/E ratio probably indicates a risk factor for cardiovascular disease in later life. The results of our present study therefore suggest that in VLBW infants, low 11ß-HSD2 activity could also contribute to the long-term metabolic and cardiovascular risks. The negative effect of cortisol on insulin sensitivity probably plays a crucial role in the association between high F/E ratio and metabolic and cardiovascular consequences (31, 32).

In SGA born children, F/E ratio is positively correlated with total and LDL cholesterol levels (5, 6). In our total group of VLBW children, we did not find significant relationships between F/E ratio and metabolic syndrome components at 2 years corrected age; however, in the subgroups of VLBW children with high glucose levels or low HDL cholesterol levels, F/E ratio was positively correlated to triglycerides and glucose, respectively. This could indicate that F/E ratio, and hence 11ß-HSD2 activity, is associated with metabolic risks in VLBW children. In the term AGA children, we found significant longitudinal relationships between F/E ratio and several metabolic parameters (cholesterol, glucose, insulin and HOMA). However, in contrast to the subgroups of VLBW children, these correlations were negative in the term AGA children. This finding is difficult to interpretate and could be related to the age of the children. The positive relation between F/E ratio and cholesterol in earlier studies was found in older children (5, 6), so it is possible that this positive relationship could not yet be consistently detected in early childhood.

We earlier showed that VLBW children treated with insulin in the first postnatal week have higher serum cortisol levels at 2 years corrected age than children treated with standard care (4). In the present study, we investigated the longitudinal differences between the two groups during early childhood and found that both cortisol and cortisone were higher in the early-insulin group compared to the standard care group. This confirms that early insulin treatment may affect the programming of the HPA axis, as we suggested earlier (4). F/E ratio was not different between the early-insulin and standard care group, so early insulin treatment itself appears to have no additional effect on 11ß-HSD2 activity.

Our study is limited by the small number of children. This could explain why we did not find higher cortisol levels in VLBW children, in contrast to other studies (26-28, 30). Cortisol, cortisone and F/E ratio should be measured in larger groups of VLBW and term born children, at the same time points, in early and later childhood, in order to confirm the results of the present study and to find out whether the higher F/E ratio persists in older children. This future study should also include measurements of several metabolic syndrome components, including blood pressure, at several time points in order to clarify the relationship between F/E ratio and metabolic parameters in both VLBW and term born children.

In conclusion, VLBW infants have higher F/E 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. In VLBW infants, early insulin treatment could affect the programming of the HPA axis, resulting in higher cortisol and cortisone levels during early childhood.

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Chapter 8

IGF-I and relation to growth in infancy and early childhood in very-low-birth-weight infants and term born infants

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ABSTRACT

Background

In very-low-birth-weight infants insulin-like growth factor I (IGF-I) plays an important role in postnatal growth restriction and is probably also involved in growth restriction in childhood. We compared IGF-I and its relation to growth in early childhood in very-low-birth-weight infants and term appropriate-for-gestational-age born infants.

Methods

We included 41 very-low-birth-weight and 64 term infants. Anthropometry was performed at all visits to the outpatient clinic. IGF-I and insulin were measured in blood samples taken at 6 months and 2 years corrected age (very-low-birth-weight children) and at 3 months, 1 and 2 years (term children).

Results

Over the first 2 years of life, growth parameters are lower in very-low-birth-weight children compared to term children, but the difference in length decreases significantly. During the first 2 years of life, IGF-I is higher in very-low-birth-weight children compared to term children. In both groups there is a significant relationship between IGF-I and (change in) length and weight over the first 2 years of life and between insulin and change in total body fat.

Conclusions

Considering the relation of IGF-I to growth and the decrease in difference in length, higher IGF-I levels in very-low-birth-weight infants in early childhood probably have an important role in catch-up growth in length.

INTRODUCTION

Insulin-like growth factor I (IGF-I) is important for fetal and postnatal growth and development. In contrast to term infants, IGF-I levels in preterm infants decrease after birth and only increase gradually during the early postnatal period (1). Studies in preterm infants show that IGF-I levels during the first postnatal weeks are positively related to early postnatal growth (2-6). IGF-I not only plays an important role in postnatal growth restriction; studies in older children (between 5 and 10 years of age) suggest that IGF-I also is involved in prolonged growth restriction in very-low-birth-weight (VLBW) infants (7, 8). IGF-I levels in mid-childhood in preterm infants compared to term born children were found lower in one study (8), but higher in another study (9).

The aim of the present study was to compare IGF-I and its relation to growth parameters in infancy and early childhood in VLBW infants (birth weight < 1500 g) to term appropriate-for-gestational-age (AGA) born infants.

METHODS

Study population

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 (10). 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 included maternal diabetes and major congenital anomalies. All infants participating in the NIRTURE trial in our neonatal intensive care unit (inclusion period from 2006 to 2007) were eligible for the present study. Therefore the sample size of the VLBW infants in the present study was determined by the number of infants we included in the NIRTURE trial.

The term infants were born between 2000 and 2005 from a low-risk population of pregnant women included in the first trimester in a prospective longitudinal study (Trophoblast study), which aimed to investigate the use of circulating trophoblast for prenatal diagnosis of pregnancy-associated diseases such as preeclampsia (11). Only term infants born AGA were included in the present study. AGA was defined as a birth weight above the 10th percentile (12). Standard deviation scores (SDS) of birth weight were calculated according to Niklasson et al. (13). Approval from the ethics committee of the VU University Medical Center was obtained.

During the inclusion period of the NIRTURE trial in our neonatal intensive care unit (21 months), 165 VLBW infants were admitted and the parents of 69 infants were approached regarding participation in the study. The most common reasons for not approaching parents were infants not requiring intensive care or no opportunity to obtain informed consent within the first 24 hours after birth. In our unit, 47 VLBW infants participated in the NIRTURE trial. Five infants died and one child was excluded because parents refused blood sampling at the follow-up visits; 41 VLBW children were included in the present study. At 2 years corrected age, one of these 41 children was lost to follow-up. Four (10%) of the VLBW children were SGA (defined as a birth weight below the 10th percentile (12)). Seventeen infants (9 male/8 female) were assigned to the early-insulin group and 24 infants (12 male/12 female) received standard neonatal care. During the first week of life, 6 infants in the standard care group were treated with insulin for 1 or 2 days because of hyperglycemia due to sepsis.

Ninety term born infants were included in the follow-up part of the Trophoblast study of whom 72 were AGA. Eight AGA children were excluded from the present study because they were lost to follow-up after the first visit at 3 months of age; 64 children were included in the present study. At 2 years of age, 6 children were lost to follow-up.

Data collection

The VLBW infants visited the outpatient clinic at expected date of delivery and at the corrected ages of 3 and 6 months, 1 year and 2 years, the term born infants at 3 months, 1 year and 2 years of age, according to the protocol of the NIRTURE trial and Trophoblast study, respectively. At each visit, anthropometry according to Dauncey et al. (14) was performed by the same trained research nurse in all children. Body weight was measured using an electronic scale to the nearest 0.1 kg, standing height was measured to the nearest 0.1 cm and all lengths and circumferences were measured using a measuring tape to the nearest 0.1 cm. Body mass index (BMI) was calculated. Total body fat was calculated according to Dauncey et al. (14) from skinfold thickness measurements and body dimensions. Standard deviation scores of weight, height, head circumference and BMI were calculated according to Dutch references (15, 16). Blood samples for IGF-I and insulin measurement were taken at 6 months and 2 years corrected age in the VLBW infants and at 3 months, 1 and 2 years of age in the term born infants, according to the specific study protocol. All blood samples were taken early in the afternoon after a fasting period of at least 3 hours. Samples were stored at -80 ^{o}C and were all analysed at the same time. Study population and data collection also have been previously described (17-19).

Assays

IGF-I in serum was measured by chemiluminescence immunoassay (Liaison, DiaSorin, Saluggia, Italy). Intra-assay coefficients of variation are 8% at both 10.3 nmol/l and 17.5 nmol/l and 9% at 23.8 nmol/l. Inter-assay coefficients of variation are 10% at 6.9 nmol/l, 7.4% at 30.8 nmol/l and 16% at 59.4 nmol/l.

Insulin in serum was measured by immunometric assay (Advia Centaur, Siemens Medical Solutions Diagnostics, Malvern, Pennsylvania, USA). Lower limit of quantitation is 10 pmol/l. Intra-assay coefficients of variation are 4% at 20 pmol/l, 3% at 500 pmol/l and 4% at 1500 pmol/l. Inter-assay coefficients of variation are 8% at 24 pmol/l and 7% at both 780 pmol/l and 3000 pmol/l.

Statistical analysis

Statistical analyses were performed using the Statistical Package of Social Sciences software for Microsoft Windows version 19 (SPSS Inc., Chicago, Illinois, USA) and Stata version 14 (StataCorp, College Station, Texas, USA). Differences in characteristics between VLBW children and term AGA children were evaluated using Student's t-test for normally distributed values and Mann-Whitney test and Wilcoxon signed-rank test for not normally distributed values.

Longitudinal differences in anthropometry, IGF-I and insulin between the two groups were analysed with linear mixed model analyses. In a first analysis, growth parameters at different time points were compared between the groups and by adding an interaction between group and time to the model, it was evaluated whether the difference between the groups changed over time. In a second analysis, the differences in IGF-I and insulin between the groups on average over time were investigated. Because both IGF-I and insulin were highly skewed to the right, both variables were log transformed before analyses. Finally, the longitudinal relationship between IGF-I and insulin on the one hand and growth parameters on the other hand was investigated. This was done for the absolute growth parameters as well as for the changes in growth parameters between subsequent time points. Group differences in the observed relationships between IGF-I and insulin and growth parameters were investigated by adding an interaction between group and IGF-I and insulin to the linear mixed models. All analyses were (if possible) adjusted for gestational age, gender and target height. For insulin levels below the limit of quantitation a value of 1 pmol/l was used. P values < 0.05 were considered as significant.

RESULTS

Table 1 shows the characteristics of the VLBW infants and term AGA infants including parental background information.

Table 1. Characteristics of the VLBW and term AGA children

	VLBW (n=41)	Term AGA (n=64)	p-value
Sex	21 M / 20 F	35 M / 29 F	0.728
Gestational age (wk)	27.9 ± 1.3	39.3 ± 1.2	< 0.001
Birth weight (g)	1059 ± 231	3529 ± 393	< 0.001
Birth weight SDS	-0.06 ± 0.9	0.3 ± 0.7	0.019
Maternal age (years)	31.3 ± 4.7	33.7 ± 4.4	0.01
Maternal weight (kg)	68.2 ± 13.7	71.7 ±13.2	0.206
Maternal smoking	5/41 (12%)	6/64 (9%)	0.747
Racial group	26 Caucasian, 10 Black, 3 Moroccan, 2 Asian	55 Caucasian, 4 Black, 5 Asian	
Highest level of parental education[a]	3 low, 18 medium, 20 high	1 low, 19 medium, 35 high, 9 unknown	
Target height SDS	0.2 ± 0.9	0.5 ± 0.9	0.181
Breast feeding	31/41 (76%)	45/64 (70%)	0.554
- duration of exclusive breast feeding (months)	3 (0-8)	3 (0-6)	0.207
- total duration of breast feeding (months)	5 (1-23)	4 (1-24)	0.924
Weight (g) at expected date of delivery[b]	3154 ± 579		< 0.001
Weight SDS at expected date of delivery[b]	-1.2 ± 1.3		< 0.001

Data are expressed as mean ± standard deviation, percentages or numbers; duration of breast feeding is presented as median (range).
VLBW infants are compared to term AGA infants.
[a]Highest level of education completed by either parent was used as an indicator of socioeconomic status and classified as low (primary school, low occupational training), medium (high school, medium occupational training) or high (high occupational training, university).
[b]Weight (SDS) at expected date of delivery of the VLBW infants was compared to birth weight (SDS) of the term AGA infants.

Anthropometry

Table 2 shows the weight (SDS), length (SDS), head circumference (SDS), BMI (SDS) and total body fat at 3 months, 1 year and 2 years of (corrected) age for the VLBW and term AGA children. Longitudinal analysis showed that on average over the first 2 years of life, all these growth parameters were significantly lower in VLBW children compared to term AGA children. The difference in length (SDS) between the VLBW children and term AGA children decreased significantly in the course of these 2 years. For the other growth parameters, the difference between the VLBW children and term AGA children increased or did not change over time.

IGF-I and insulin

Table 3 shows IGF-I and insulin for the VLBW and term AGA children. Longitudinal analysis showed that on average over the first 2 years of life, IGF-I was significantly higher

Table 2. Anthropometry of the VLBW (n = 41) and term AGA (n = 64) children

	VLBW	Term AGA	VLBW	Term AGA	VLBW	Term AGA	Difference[a]	p-value[a]	Interaction with time[b]
	At 3 months (corrected) age		*At 1 year (corrected) age*		*At 2 years (corrected) age*				
Weight (kg)	5.6 ± 0.9	6.2 ± 0.6	9.1 ± 1.2	10.1 ± 1.0	11.5 ± 1.3	13.0 ± 1.5	-1.3 (-1.9 to -0.8)	< 0.001	negative
Weight SDS	-0.6 ± 1.4	0.5 ± 0.7	-0.9 ± 1.1	0.1 ± 0.8	-0.9 ± 1.0	0.1 ± 0.9	-1.0 (-1.3 to -0.7)	< 0.001	no
Length (cm)	58.2 ± 2.7	61.4 ± 2.0	74.5 ± 2.9	76.5 ± 2.4	86.0 ± 3.7	87.7 ± 3.3	- 3.7 (-6.1 to -1.5)	0.001	positive
Length SDS	-1.1 ± 1.1	0.4 ± 0.7	-0.6 ± 1.0	0.1 ± 0.8	-0.7 ± 1.1	-0.3 ± 0.9	-0.9 (-1.2 to -0.5)	< 0.001	positive
Head circumference (cm)	40.4 ± 1.5	41.3 ± 1.3	46.1 ± 1.6	47.2 ± 1.5	48.3 ± 1.7	49.4 ± 1.5	-1.3 (-2.1 to -0.6)	< 0.001	no
Head circumference SDS	-0.1 ± 1.0	0.6 ± 0.8	-0.4 ± 0.9	0.4 ± 0.9	-0.2 ± 0.9	0.4 ± 0.9	-0.7 (-1.0 to -0.4)	< 0.001	no
BMI (kg/m2)	16.3 ± 1.8	16.4 ± 1.2	16.3 ± 1.5	17.2 ± 1.3	15.5 ± 1.5	16.9 ± 1.1	-0.6 (-1.1 to -0.2)	0.004	negative
BMI SDS	0.1 ± 1.3	0.3 ± 0.9	-0.7 ± 1.1	0.1 ± 0.9	-0.7 ± 1.2	0.5 ± 0.8	-0.6 (-1.0 to -0.3)	< 0.001	negative
Total body fat (kg)	1.0 ± 0.5	1.3 ± 0.3	1.6 ± 0.6	2.3 ± 0.7	1.8 ± 0.6	2.6 ± 1.0	-0.5 (-0.7 to -0.4)	< 0.001	negative
TBF/weight	0.18 ± 0.06	0.20 ± 0.04	0.18 ± 0.06	0.22 ± 0.05	0.16 ± 0.05	0.20 ± 0.06	-0.03 (-0.05 to -0.015)	< 0.001	no

Data are expressed as mean ± standard deviation.

[a]Differences and p-values were based on the comparison between VLBW children and term AGA children on average over time adjusted for gender.

[b]Negative interaction indicates that the differences between the groups became stronger over time; positive interaction indicates that the differences between the groups became less strong over time.

Table 3. IGF-I and insulin

	VLBW (n=41)	Term AGA (n=64)
At 3 months (corrected) age		
IGF-I (nmol/l)		7.7 (3.7-14.4)
At 6 months (corrected) age		
IGF-I (nmol/l)	10.2 (2.3-30.9)	
Insulin (pmol/l)	23.0 (1.0-256.7)	
At 1 year (corrected) age		
IGF-I (nmol/l)		6.8 (2.0-19.0)
Insulin (pmol/l)		15.7 (1.0-179.4)
At 2 years (corrected) age		
IGF-I (nmol/l)	11.6 (3.5-26.8)	9.5 (4.2-21.0)
Insulin (pmol/l)	21.0 (1.0-190.9)	17.9 (1.0-181.1)
Difference between the VLBW and term AGA children on average over time adjusted for gender		
IGF-I (nmol/l)	1.36[a] (1.19 to 1.56); $p < 0.001$	
Insulin (pmol/l)	1.37[a] (0.86 to 2.17); $p = 0.182$	

Data are expressed as median and range.
[a]Difference expressed as a ratio (VLBW compared to term AGA; for example, VLBW children have 1.36 higher concentration of IGF-I compared to term AGA children).

in VLBW children compared to term AGA children. Insulin did not differ between the VLBW and term AGA children over time. In the VLBW children, we also compared the early-insulin group to the standard care group and the SGA born children to the AGA born children and did not find significant differences. In VLBW children, IGF-I at 2 years corrected age was significantly higher than at 6 months corrected age ($p = 0.023$). In term AGA children, IGF-I at 2 years of age was significantly higher than at 1 year of age ($p < 0.001$).

Relation of IGF-I and insulin to growth parameters

Tables 4 and 5 show the longitudinal relationship over the first 2 years of life between IGF-I and insulin on the one hand and growth parameters on the other hand. In table 4 the relationship with the absolute growth parameters is shown and in table 5 the relationship with the changes in growth parameters between subsequent time points. In both groups, there was a significant relationship between IGF-I and (change in) length and weight over the first 2 years of life and between IGF-I and total body fat. The relationship between IGF-I and (change in) head circumference and BMI SDS was only significant in the term AGA children. There was no significant relationship between insulin and (change in) length and weight over the first 2 years of life, but only between insulin and change in total body fat in both groups.

Table 4. Longitudinal relationship between IGF-I and insulin and growth parameters

	VLBW	Term AGA
IGF-I[a]		
Weight	0.14 (0.04 to 0.24); p = 0.004	0.23 (0.15 to 0.32); p < 0.001
Weight SDS	0.05 (0.01 to 0.10); p = 0.01	0.05 (0.01 to 0.08); p = 0.004
Length	0.58 (0.15 to 1.02); p = 0.009	0.75 (0.46 to 1.04); p < 0.001
Length SDS	0.06 (0.02 to 0.10); p = 0.007	-0.02 (-0.06 to 0.01); p = 0.20
Head circumference	0.04 (-0.08 to 0.17); p = 0.49	0.15 (0.06 to 0.24); p = 0.002
Head circumference SDS	0.02 (-0.01 to 0.04); p = 0.24	0.002 (-0.03 to 0.03); p = 0.90
BMI	0.01 (-0.05 to 0.08); p = 0.69	0.04 (-0.02 to 0.09); p = 0.20
BMI SDS	0.02 (-0.03 to 0.07); p = 0.41	0.08 (0.04 to 0.12); p < 0.001
Total body fat	0.03 (0.01 to 0.06); p = 0.006	0.06 (0.02 to 0.09); p = 0.002
Insulin[b]		
Weight	-0.01 (-0.02 to 0.003); p = 0.16	0.003 (-0.007 to 0.01); p = 0.56
Weight SDS	-0.001 (-0.01 to 0.004); p = 0.75	0.003 (-0.001 to 0.006); p = 0.09
Length	-0.05 (-0.11 to 0.01); p = 0.12	0.01 (-0.03 to 0.04); p = 0.65
Length SDS	-0.01 (-0.01 to 0.00); p = 0.05	0.006 (0.002 to 0.01); p = 0.003
Head circumference	-0.01 (-0.03 to 0.003); p = 0.13	-0.002 (-0.012 to 0.009); p = 0.71
Head circumference SDS	-0.002 (-0.01 to 0.00); p = 0.06	0.001 (-0.02 to 0.004); p = 0.55
BMI	0.01 (-0.002 to 0.01); p = 0.19	-0.001 (-0.01 to 0.005); p = 0.70
BMI SDS	0.003 (-0.003 to 0.01); p = 0.27	-0.001 (-0.006 to 0.003); p = 0.62
Total body fat	0.002 (-0.001 to 0.005); p = 0.26	0.008 (0.004 to 0.01); p < 0.001

All analyses were adjusted for target height, gestational age and gender. For example, in VLBW children a difference of 1 unit in IGF-I is associated with a difference of 0.14 units in weight on average over time.
[a]Regarding IGF-I, there were significant group differences for length SDS and BMI SDS.
[b]Regarding insulin, there were significant group differences for length SDS.

DISCUSSION

The present study shows that during the first 2 years of life, VLBW infants have significantly higher IGF-I levels than term AGA children. In both VLBW and term born children, there is a significant relationship between IGF-I and (change in) length and weight over the first 2 years of life.

The majority of studies concerned with IGF-I levels in VLBW infants have focused on the early postnatal period. Studies of IGF-I levels in older VLBW children are limited. Patel et al. (20) showed a significant correlation between IGF-I output and both weight and length, during the first 2 years in infants born between 24 and 33 weeks gestational age, which is in accordance with our results. They did not compare IGF-I levels with a control group of term born children. Two previous studies in mid-childhood suggest that IGF-I is involved in prolonged growth restriction in preterm born infants. Kwinta et

Table 5. Longitudinal relationship between IGF-I and insulin and changes in growth parameters

	VLBW	Term AGA
IGF-I[a]		
Δ Weight	0.03 (0.003 to 0.06); p = 0.03	-0.01 (-0.06 to 0.04); p = 0.63
Δ Weight SDS	0.01 (-0.01 to 0.04); p = 0.50	0.06 (0.02 to 0.10); p < 0.001
Δ Length	0.15 (0.03 to 0.26); p = 0.01	0.15 (-0.30 to -0.004); p = 0.04
Δ Length SDS	0.01 (-0.02 to 0.04); p = 0.36	0.01 (-0.02 to 0.05); p = 0.54
Δ Head circumference	-0.02 (-0.05 to 0.02); p = 0.32	-0.19 (-.29 to -0.09); p < 0.001
Δ Head circumference SDS	0.009 (-0.01 to 0.03); p = 0.39	0.03 (-0.0036 to 0.06); p = 0.08
Δ BMI	-0.03 (-0.08 to 0.02); p = 0.25	-0.03 (-0.10 to 0.04); p = 0.36
Δ BMI SDS	-0.001 (-0.04 to 0.04); p = 0.95	0.06 (0.02 to 0.11); p = 0.01
Δ Total body fat	0.008 (-0.01 to 0.03); p = 0.47	-0.02 (-0.06 to 0.02); p = 0.23
Insulin[b]		
Δ Weight	-0.002 (-0.19 to 0.03); p = 0.16	0.005 (-0.001 to 0.01); p = 0.13
Δ Weight SDS	0.001 (-0.002 to 0.005); p = 0.50	0001 (-0.004 to 0.006); p = 0.64
Δ Length	-0.01 (-0.03 to 0.004); p = 0.14	0.02 (-0.001 to 0.03); p = 0.06
Δ Length SDS	0.001 (-0.003 to 0.005); p = 0.66	0.003 (-0.02 to 0.04); p = 0.54
Δ Head circumference	0.002 (-0.003 to 0.006); p = 0.41	0.004 (-0.008 to 0.02); p = 0.50
Δ Head circumference SDS	0.001 (-0.002 to 0.004); p = 0.59	0.0003 (-0.003 to 0004); p = 0.87
Δ BMI	0.004 (-0.003 to 0.01); p = 0.28	0.000 (-0.008 to 0.008); p = 0.99
Δ BMI SDS	0.001 (-0.004 to 0.007); p = 0.66	-0.001 (-0.01 to 0.004); p = 0.66
Δ Total body fat	0.004 (0.001 to 0.006); p = 0.02	0.007 (0.002 to 0.01); p = 0.003

All analyses were adjusted for target height, gestational age and gender. For example, in VLBW children a difference of 1 unit in IGF-I is associated with a change of 0.03 units in weight between two subsequent measurements on average over time.

[a]Regarding IGF-I, there were significant group differences for Δ weight SDS, Δ head circumference, Δ BMI SDS and Δ total body fat.

[b]Regarding insulin, there were significant group differences for Δ weight and Δ length.

al. (7) showed that 7-year-old extremely-low-birth-weight children with short stature have lower IGF-I levels than those with normal stature. Cutfield et al. (8) showed that VLBW children between 5 and 10 years of age did not reach their mid-parental height SDS and had low IGF-I levels compared to term born AGA children.

Our present study confirms the role of IGF-I in childhood growth in VLBW infants. The VLBW children in our study were shorter than term AGA children, but the difference in length between the VLBW and term AGA children decreased during the first 2 years of life. Therefore our finding of significantly higher IGF-I levels during the first 2 years of life in VLBW children compared to term AGA children, together with the longitudinal relationship between IGF-I and growth, suggests that IGF-I has an important role in the catch-up growth in length in early childhood in VLBW infants. Our results are in accordance with the study of Rajaram et al. (21), showing higher IGF-I levels in preterm infants

compared to term born infants from 2 through 12 months of (corrected) age. Kistner et al. (9) found higher IGF-I levels in 9-year-old preterm born children compared to term born controls; as the preterm children were significantly shorter than the term born children, they hypothesize that preterm born children may have reduced sensitivity in the IGF-I receptors.

During early postnatal life and at 1 year corrected age, IGF-I levels in preterm infants are positively correlated to head circumference and brain volume (22-24). In the present study, we only found a significant relationship between IGF-I and head circumference in the term AGA children. The absence of this relationship in the VLBW children is probably caused by the small size of the group. We could not explain the inverse relation between IGF-I and change in head circumference in the term AGA children.

In the study of van de Lagemaat et al. (25) in preterm infants between term age and 6 months corrected age, both IGF-I and insulin were correlated to growth parameters. At 6 months corrected age, the correlation between insulin and IGF-I had disappeared, indicating the shift from insulin dependency to growth hormone (GH) dependency of IGF-I with increasing postnatal age. In the present study, we did not measure insulin before 6 months of (corrected) age; this explains why we could not explore any relationship between insulin and growth in our study. We hypothesize that the association between insulin and total body fat could be caused by the influence of body composition on insulin sensitivity. We found some differences between the VLBW and term born children in the relationships between IGF-I and insulin and (changes in) growth parameters (tables 4 and 5), but the groups are too small to draw any conclusions from these differences.

The most important limitation of our study is the small number of children. The results have to be confirmed in larger groups of VLBW children. Including serial IGF-I measurements from the early postnatal period until late childhood in both VLBW infants and term born controls at the same time points, could contribute to the knowledge about the role of IGF-I in growth of VLBW infants during childhood, its relation to catch-up growth and about the meaning of the higher IGF-I levels in preterm born children compared to term born children. Another limitation is the amount of statistical tests performed. Because of this, individual significant results should be interpreted with caution.

In conclusion, as IGF-I is related to growth in length, and the difference in length compared to term born children decreases during early childhood, the higher IGF-I levels in VLBW infants during the first 2 years of life probably have an important role in catch-up growth in length.

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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.

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Summary

SUMMARY

Preterm birth interrupts the normal fetal growth and developmental processes of many organs and regulating systems. The combination of preterm birth itself, postnatal morbidity and stress and impaired postnatal growth could have consequences for later health.

Preterm born children have impaired growth in early postnatal life. This period corresponds with the last trimester of pregnancy and the impaired postnatal growth might therefore result in comparable adaptations as in third trimester intra-uterine growth restriction. These changes in structure and metabolism (programming) are functional for survival in the adverse intra-uterine or extra-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/).

It is unknown whether the long-term endocrine and metabolic consequences of preterm birth and programming in the postnatal period are already detectable in early childhood. 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, the effects of early insulin therapy on the neuroendocrine axes and on the components of the metabolic syndrome were also investigated. The background and aims of this thesis are further addressed in **chapter 1**.

Chapter 2 and 3 describe the postnatal activation of the HPG axis in male and female VLBW infants. Serial measurements of gonadotropins and testosterone/estradiol levels, by making use of urine samples, provided an accurate description of the postnatal activation of the HPG axis without the burden of frequent blood sampling. In VLBW boys, levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) showed a peak at a mean postnatal age of 1 to 4 weeks (mean postmenstrual age of 30 to 32 weeks). Testosterone levels did not show a significant peak, but decreased with increasing age; this decrease was faster in infants receiving early insulin therapy compared to those receiving standard care. In VLBW girls, levels of FSH and LH showed a peak at a mean postmenstrual age of 32 weeks (postnatal age of 4 weeks) and estradiol levels were highest shortly after birth.

Chapter 4 describes the prevalence of the components of the metabolic syndrome in VLBW infants at the corrected age of 2 years, using reference values published earlier. The majority of the VLBW infants already had one or more components of the metabolic syndrome at that age. 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). 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 long-term benefits for some of the components of the metabolic syndrome.

Chapter 5 compares the components of the metabolic syndrome in VLBW infants at the corrected age of 2 years to those in 2-year-old term appropriate-for-gestational-age (AGA) born children from our own population. At 2 years corrected age, VLBW children had higher glucose levels than term AGA children. This suggests that VLBW children already have reduced insulin sensitivity at 2 years corrected age.

In **chapter 6**, the relation between cortisol levels and blood pressure is investigated. At 2 years corrected age, cortisol levels were positively correlated to blood pressure in VLBW boys. This suggests that programming of the HPA axis may contribute to the high prevalence of raised blood pressure in early childhood in VLBW infants. This chapter also confirms the reliability of salivary cortisol measurements, which are preferable to serum measurements because of the non-invasive sample collection.

Chapter 7 compares serum cortisol and cortisone levels and cortisol/cortisone ratio in VLBW infants to term AGA born infants. During the first 2 years of life, cortisol/cortisone ratio was higher in VLBW children compared to term children, suggesting lower activity of 11ß-hydroxysteroid dehydrogenase type 2 (11ß-HSD2). Considering the relationship between cortisol/cortisone ratio and metabolic syndrome components, lower 11ß-HSD2 activity probably contributes to the long-term metabolic and cardiovascular risks of VLBW infants. Over the first 2 years of life, both cortisol and cortisone were higher in VLBW children treated with insulin in the first postnatal week compared to children in the standard care group. This suggests that early insulin treatment may affect the programming of the HPA axis.

Chapter 8 compares IGF-I and its relation to growth in VLBW infants to term AGA born infants. During the first 2 years of life, IGF-I levels were higher in VLBW children com-

pared to term children. In both VLBW and term born infants, IGF-I levels were related to (change in) length and weight over the first 2 years of life. The difference in length between VLBW and term born infants decreased over the first 2 years of life, suggesting that higher IGF-I levels in VLBW infants may have an important role in catch-up growth in length in early childhood.

Chapter 9 discusses the results of the studies in this thesis and the implications of the endocrine and metabolic changes in early childhood in VLBW infants. The higher prevalence of metabolic syndrome components and the risk of cardiovascular disease have implications for life style and follow-up; this should be taken into account by healthcare professionals, parents and the VLBW children during their entire lives. Several suggestions for future research are described. Finally, the development of interventions that may reduce the metabolic risks in later life of VLBW infants is discussed.

Nederlandse samenvatting

NEDERLANDSE SAMENVATTING

De groei en de ontwikkeling van diverse orgaansystemen en regelmechanismen in de foetus tijdens de periode in de baarmoeder worden verstoord door vroeggeboorte. Deze verstoring kan op de lange termijn gevolgen hebben voor de gezondheid. Ook slechte groei en doorgemaakte ziekten in de periode kort na de geboorte kunnen hier een rol in spelen. Te vroeg geboren kinderen (prematuren) groeien in de eerste maanden na de geboorte minder hard dan dat een foetus normaal groeit tijdens de vergelijkbare periode in de baarmoeder (het laatste trimester van de zwangerschap). De verminderde groei van een prematuur kort na de geboorte zou tot dezelfde aanpassingen kunnen leiden als de aanpassingen die optreden bij verminderde groei van een foetus in de baarmoeder tijdens het laatste trimester van de zwangerschap (dit wordt intra-uteriene groei restrictie genoemd). Deze aanpassingen veroorzaken permanente veranderingen in de structuur en functie van orgaansystemen ('programmering'). Op korte termijn hebben deze aanpassingen voordelen en vergroten ze de overlevingskansen in de ongunstige omstandigheden; later in het leven zijn deze aanpassingen echter geassocieerd met niet-overdraagbare chronische ziekten, zoals hypertensie, hart- en vaatziekten en diabetes mellitus type 2. Deze associaties werden bekend als de Barker hypothese en staan tegenwoordig bekend als de Developmental Origins of Health and Disease (DOHaD) hypothese.

Het is reeds aangetoond dat vroeggeboorte leidt tot veranderingen in de hormoonhuishouding en het metabolisme op volwassen leeftijd. Het is echter onbekend of deze gevolgen van vroeggeboorte en programmering al op de peuterleeftijd aantoonbaar zijn. In dit proefschrift worden deze endocriene en metabole gevolgen beschreven bij jonge, prematuur geboren kinderen. Een aantal hormonale regelmechanismen (endocriene assen) en de risicofactoren van het metabool syndroom werden bestudeerd tijdens de eerste twee levensjaren van prematuur geboren kinderen met een geboortegewicht onder de 1500 gram (VLBW = very-low-birth-weight). De endocriene assen die werden bestudeerd zijn de hypothalamus-hypofyse-gonaden (HPG) as (stuurt de geslachtsklieren aan), de hypothalamus-hypofyse-bijnier (HPA) as (stuurt de hormonale reactie op stress aan) en de groeihormoon/insuline-achtige groeifactor I (GH/IGF-I) as (regelt de lengtegroei). Het metabool syndroom bestaat uit een combinatie van risicofactoren en gaat gepaard met een verhoogde kans op hart- en vaatziekten en diabetes mellitus type 2. Risicofactoren van het metabool syndroom zijn obesitas, verhoogde bloeddruk, verhoogde triglyceridenwaarde, verlaagde HDL cholesterolwaarde en verhoogde glucosewaarde. De bestudeerde VLBW kinderen namen eerder deel aan een onderzoek naar de effecten van behandeling met insuline in de eerste week na de geboorte (NIRTURE trial = Neonatal Insulin Replacement Therapy in Europe trial); derhalve werden ook de effecten van vroege insulinebehandeling op de genoemde endocriene

assen en op de risicofactoren van het metabool syndroom bestudeerd. De achtergrond van dit proefschrift wordt verder besproken in **hoofdstuk 1**.

In de **hoofdstukken 2 en 3** wordt de activatie van de HPG as kort na de geboorte bij VLBW jongens en meisjes beschreven. Het gebruik van herhaalde metingen van gonadotrofinen (follikelstimulerend hormoon (FSH) en luteïniserend hormoon (LH)) en testosteron/estradiol in urinemonsters, maakte het mogelijk om deze activatie van de HPG as te bestuderen, zonder herhaaldelijk bloed af te hoeven nemen. Bij VLBW jongens was er zowel voor FSH als LH een piek aantoonbaar op een mediane kalenderleeftijd van 1 tot 4 weken (mediane postmenstruele leeftijd van 30 tot 32 weken). Testosteron daalde bij toename van de leeftijd, er was geen piek aantoonbaar. Testosteron daalde sneller in de groep met vroege insulinebehandeling, vergeleken met de groep met standaard behandeling. Bij VLBW meisjes was er voor FSH en LH een piek aantoonbaar op een mediane postmenstruele leeftijd van 32 weken (kalenderleeftijd van 4 weken). Estradiol concentraties waren kort na de geboorte maximaal.

In **hoofdstuk 4** worden de risicofactoren van het metabool syndroom beschreven bij VLBW kinderen op de gecorrigeerde leeftijd van 2 jaar. De risicofactoren werden beoordeeld aan de hand van eerder gepubliceerde referentiewaarden. Bij een meerderheid van de VLBW kinderen waren op 2-jarige leeftijd al een of meer risicofactoren van het metabool syndroom aanwezig. Een verhoogde bloeddruk kwam het meeste voor: 63% van de kinderen had een verhoogde systolische en/of diastolische bloeddruk (≥ p90 voor leeftijd, geslacht en lengte). Verder had ongeveer een derde van de VLBW kinderen een verhoogde triglyceridenwaarde (≥ 0.98 mmol/l) en een derde had een verlaagde HDL cholesterolwaarde (≤ 1.03 mmol/l). VLBW kinderen in de groep met vroege insulinebehandeling hadden gemiddeld een lagere triglyceridenwaarde dan VLBW kinderen in de groep met standaard behandeling. In de subgroep van kinderen met een lage body mass index (BMI SDS < 0) op 2-jarige leeftijd, was de gemiddelde HDL cholesterolwaarde hoger bij kinderen met vroege insulinebehandeling vergeleken met kinderen met standaard behandeling. Deze bevindingen suggereren dat insulinebehandeling in de eerste week na de geboorte een gunstig effect zou kunnen hebben op een aantal van de risicofactoren van het metabool syndroom later in het leven.

In **hoofdstuk 5** worden de risicofactoren van het metabool syndroom bij VLBW kinderen op de gecorrigeerde leeftijd van 2 jaar vergeleken met deze risicofactoren bij 2-jarige kinderen, geboren na een normale zwangerschapsduur (a terme geboren) en met een normaal geboortegewicht (AGA = appropriate-for-gestational-age: geboortegewicht ≥ p10). Op de leeftijd van 2 jaar was de gemiddelde glucosewaarde hoger bij de VLBW kin-

deren vergeleken met de a terme geboren kinderen. Dit suggereert dat VLBW kinderen al op de leeftijd van 2 jaar een lagere insulinegevoeligheid hebben.

In **hoofdstuk 6** wordt de relatie tussen cortisolwaarden en bloeddruk onderzocht. Op de gecorrigeerde leeftijd van 2 jaar was er een positieve correlatie tussen cortisolwaarden en bloeddruk bij VLBW jongens. Dit suggereert dat programmering van de HPA as, die de productie van cortisol regelt, een rol zou kunnen spelen bij de verhoogde bloeddruk die al op de vroege kinderleeftijd aantoonbaar is bij een deel van de VLBW kinderen. Ook wordt in dit hoofdstuk bevestigd dat de bepaling van cortisol in speekselmonsters betrouwbaar is. Omdat afname van speeksel veel minder belastend is, heeft deze methode de voorkeur boven bepaling in bloed, zeker bij kinderen.

In **hoofdstuk 7** worden cortisol, cortisone en cortisol/cortisone ratio in bloed vergeleken tussen VLBW kinderen en a terme geboren AGA kinderen. Gedurende de eerste twee levensjaren hadden VLBW kinderen een hogere cortisol/cortisone ratio dan a terme geboren AGA kinderen. Het enzym 11ß-hydroxysteroid dehydrogenase type 2 (11ß-HSD2) zet cortisol om in het inactieve cortisone; een hogere cortisol/cortisone ratio kan dan ook duiden op een lagere activiteit van 11ß-HSD2 bij VLBW kinderen. Ook was er een relatie aantoonbaar tussen cortisol/cortisone ratio en risicofactoren van het metabool syndroom. Deze bevindingen suggereren dat een lagere 11ß-HSD2 activiteit zou kunnen bijdragen aan de metabole en cardiovasculaire gevolgen van vroeggeboorte later in het leven. VLBW kinderen in de groep met vroege insulinebehandeling hadden hogere cortisol- en cortisonewaarden gedurende de eerste twee levensjaren dan VLBW kinderen in de groep met standaard behandeling. Dit suggereert dat insulinebehandeling in de eerste week na de geboorte de programmering van de HPA as zou kunnen beïnvloeden.

In **hoofdstuk 8** worden IGF-I en de relatie met groei gedurende de eerste twee levensjaren vergeleken tussen VLBW kinderen en a terme geboren AGA kinderen. VLBW kinderen hadden hogere IGF-I waarden dan a terme geboren AGA kinderen. In beide groepen werd gedurende de eerste twee levensjaren een relatie gevonden tussen IGF-I waarden en (toename in) lengte en gewicht. Gedurende deze hele periode waren VLBW kinderen gemiddeld kleiner dan a terme geboren AGA kinderen, het verschil in lengte tussen beide groepen nam echter af in de loop van deze twee jaren. Deze afname in het verschil in lengte suggereert dat de hogere IGF-I waarden bij VLBW kinderen een belangrijke rol kunnen spelen bij het optreden van inhaalgroei op de vroege kinderleeftijd.

Hoofdstuk 9 geeft een overzicht van de bevindingen en conclusies van de onderzoeken beschreven in dit proefschrift. Tevens wordt er een aanzet gegeven voor de implicaties van deze resultaten voor de praktijk. De risicofactoren van het metabool syndroom

zijn bij VLBW kinderen al op jonge leeftijd aantoonbaar. Aanbevolen wordt dat ouders, en later ook de VLBW kinderen zelf, worden voorgelicht over het risico op hart- en vaatziekten en adviezen krijgen over leefstijl en voeding. Betrokken artsen hebben een belangrijke rol in deze voorlichting en screening, niet alleen op de kinderleeftijd, maar ook tijdens de volwassenheid. Daarnaast worden er aanbevelingen gegeven voor toekomstig onderzoek. Dit hoofdstuk sluit af met de bespreking van mogelijke vroege interventies met als doel om de nadelige endocriene en metabole gevolgen van vroeggeboorte gunstig te beïnvloeden, of zelfs te voorkomen.

Abbreviations

ABBREVIATIONS

11ß-HSD2	11ß-hydroxysteroid dehydrogenase type 2
AGA	appropriate-for-gestational-age
BMI	body mass index
BP	blood pressure
F/E	cortisol/cortisone
FSH	follicle-stimulating hormone
GH	growth hormone
HDL	high-density lipoprotein
HOMA	homeostatic model assessment
HPA	hypothalamic-pituitary-adrenal
HPG	hypothalamic-pituitary-gonadal
IGF-I	insulin-like growth factor I
IUGR	intra-uterine growth restriction
LDL	low-density lipoprotein
LH	luteinizing hormone
NIRTURE	Neonatal Insulin Replacement Therapy in Europe
SDS	standard deviation score
SGA	small-for-gestational-age
TBF	total body fat
VLBW	very-low-birth-weight

Publications

PUBLICATIONS

This thesis

de Jong M, Cranendonk A, Twisk JW, van Weissenbruch MM. Cortisol and cortisone in early childhood in very-low-birth-weight infants and term born infants. Submitted.

de Jong M, Cranendonk A, Twisk JW, van Weissenbruch MM. IGF-I and relation to growth in infancy and early childhood in very-low-birth-weight infants and term born infants. PLoS One 2017;12(2):e0171650.

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NIRTURE trial

Beardsall K, Vanhaesebrouck S, Frystyk J, Ogilvy-Stuart AL, Vanhole C, van Weissenbruch M, Midgley P, Thio M, Cornette L, Gill B, Ossuetta I, Iglesias I, Theyskens C, **de Jong M**, Ahluwalia JS, de Zegher F, Dunger DB. Relationship between insulin-like growth factor I levels, early insulin treatment, and clinical outcomes of very low birth weight infants. J Pediatr 2014;164(5):1038-44.

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Curriculum vitae

CURRICULUM VITAE

Miranda de Jong werd geboren op 3 augustus 1973 in Rotterdam. Op 5-jarige leeftijd verhuisde ze met haar ouders en broer naar Houten. In 1991 behaalde ze het vwo-diploma aan het Dr. F.H. de Bruijne Lyceum te Utrecht. Aansluitend startte ze met de studie geneeskunde aan de Erasmus Universiteit te Rotterdam. In 1995 deed ze een onderzoeksstage bij de afdeling neonatologie van het Sophia Kinderziekenhuis te Rotterdam, onder begeleiding van Prof. dr. L.J.I. Zimmermann. In 1996 volgde ze een klinische stage in The Hospital for Sick Children te Toronto, Canada. Het doctoraalexamen behaalde ze in 1996 en het artsexamen in 1998.

Na werkzaam te zijn geweest als arts-assistent niet in opleiding op achtereenvolgens de afdeling kindergeneeskunde van het Sophia Kinderziekenhuis te Rotterdam en de afdeling kindergeneeskunde van het VU medisch centrum te Amsterdam, startte ze in 2000 met de opleiding tot kinderarts in het VU medisch centrum (opleider Prof. dr. J.J. Roord). Het niet-academisch gedeelte van de opleiding vond plaats in het St. Elisabeth Ziekenhuis te Tilburg (opleider Dr. J.M.Th. Draaisma).

In 2004 begon ze, tijdens het laatste half jaar van de opleiding kindergeneeskunde, met het fellowship neonatologie, ook in het VU medisch centrum (opleider Prof. dr. W.P.F. Fetter). Tijdens dit fellowship startte ze met het onderzoek dat tot dit proefschrift heeft geleid.

Na afronding van de opleiding neonatologie in 2007 bleef ze nog twee jaar als staflid werkzaam op de afdeling neonatologie van het VU medisch centrum. Sinds 2009 werkt ze als kinderarts-neonatoloog in het Albert Schweitzer ziekenhuis te Dordrecht. Voor het voortzetten van het promotietraject bleef ze daarnaast verbonden aan de afdeling neonatologie van het VU medisch centrum.

Dankwoord

DANKWOORD

Het is zover!

Jarenlang was promoveren het doel in de verte, waar ik stap voor stap naar toe aan het werken was. De stapjes waren niet altijd even groot, maar dankzij de hulp en steun van diverse mensen om me heen is het gelukt om steeds in de juiste richting door te blijven stappen. Een aantal van deze mensen wil ik graag in het bijzonder noemen.

Om te beginnen mijn eerste promotor, Prof. dr. M.M. van Weissenbruch. Beste Mirjam, tijdens mijn fellowship betrok je me bij de multicenter NIRTURE studie en ontstonden daaruit de ideeën voor de onderzoeken in dit proefschrift. Bij mijn vertrek uit het VUmc waren er weliswaar veel data verzameld en lagen de vriezers vol met urine-, speeksel- en bloedmonsters van 'onze' NIRTURE kinderen, maar was er vooral nog veel werk aan de winkel. Toch was je bereid het promotietraject met mij te vervolgen. Ik wil je vooral bedanken voor het vertrouwen dat je in me hebt gehouden, ook in periodes waarin mijn wetenschappelijke output wat minder was. Fijn dat er, ondanks al jouw andere werkzaamheden, altijd een moment te vinden was om van gedachten te wisselen, zodat ik weer vooruit kon. Ook je enthousiasme bij elke bereikte stap werkte voor mij zeer motiverend. Ik ben heel blij dat je nu als professor ook officieel mijn promotor bent!

Mijn tweede promotor, Prof. dr. H.N. Lafeber. Beste Harrie, fijn dat ik je altijd kon raadplegen en dank voor je bereidheid om op cruciale momenten mee te denken. Ook tijdens mijn opleiding tot kinderarts en neonatoloog stond je deur altijd voor mij open.

De eerste fase van mijn promotietraject bestond uit het includeren van te vroeg geboren kinderen in het NIRTURE onderzoek. Dit moest binnen 24 uur na de geboorte gebeuren. Mijn speciale dank gaat dan ook uit naar alle ouders die in deze emotionele periode toestemming hebben gegeven om hun pasgeboren kind(eren) te laten deelnemen aan dit onderzoek. Ook de inzet van de neonatologen, fellows en verpleegkundigen van de afdeling neonatologie van het VUmc was onmisbaar om het includeren en de behandeling met insuline goed te laten verlopen. Veel dank daarvoor!

Na ontslag uit het ziekenhuis werden de kinderen diverse malen teruggezien op de polikliniek en vervolgd tot de leeftijd van 2 jaar. Hierbij heeft Anneke Cranendonk als researchverpleegkundige een onmisbare rol gespeeld. Beste Anneke, jouw gestructureerde organisatie en goede contacten met de ouders zorgden ervoor dat de kinderen op de juiste tijdstippen werden teruggezien en dat de follow-up bijna compleet was. Dank je wel voor het verzamelen van alle gegevens, verrichten van alle metingen, opvangen van urineporties, (laten) afnemen van speeksel- en bloedmonsters en blijven motiveren van de ouders.

De medewerkers van het Endocrinologisch Laboratorium van het VUmc wil ik bedanken voor alle bepalingen die zij hebben verricht in de urine-, speeksel- en bloedmonsters. Dr. A.C. Heijboer, beste Annemieke, dank voor het steeds weer meedenken over

de mogelijkheden (en assays) om onze plannen ook uitvoerbaar te maken. Geweldig dat jullie, ondanks de vaak kleine monstervolumes, zoveel verschillende bepalingen hebben kunnen doen. Beste Josien Dijkstra-Lagemaat, dank ook voor je praktische hulp bij het voorbereiden en aanleveren van de monsters.

Prof. dr. J.W.R. Twisk, beste Jos, dank voor je hulp bij de statistiek. Fijn dat je de longitudinale analyses voor ons hebt uitgevoerd en de resultaten daarvan voor mij begrijpelijk wist te maken.

De leden van de leescommissie, Prof. dr. C.B. Lambalk, Prof. dr. L.J.I. Zimmermann, Prof. dr. J.M. Wit, Prof. dr. F. van Bel, Dr. M.J.J. Finken en Dr. A.C. Heijboer, wil ik bedanken voor de beoordeling van mijn proefschrift. Prof. dr. L.J.I. Zimmermann, beste Luc, onder jouw hoede deed ik als student in het Sophia Kinderziekenhuis de eerste ervaringen op met het doen van wetenschappelijk onderzoek, presenteren van de resultaten op congressen en het schrijven van een artikel. Ook woonde ik voor het eerst een promotie bij: die van jou. Ik vind het dan ook ontzettend leuk dat je deel uitmaakt van de promotiecommissie. Prof. dr. J.M. Wit, dank u voor de genomen moeite om uw opmerkingen en suggesties persoonlijk met mij door te nemen.

Dr. K. Beardsall, dear Kathy, thank you for the pleasant collaboration in the NIRTURE trial.

Mijn (oud-)collega's van de vakgroep Kindergeneeskunde van het Albert Schweitzer ziekenhuis ben ik heel dankbaar voor de periode waarin ik een dagdeel 'werktijd' mocht besteden aan mijn promotie. Deze extra tijd heeft me erg geholpen om belangrijke stappen vooruit te kunnen zetten. Ook wil ik jullie bedanken voor de fijne werksfeer en voor jullie aanhoudende interesse in mijn vorderingen. Ik ben blij dat ik mijn volmondig 'Ja!' als antwoord op de vraag tijdens mijn sollicitatiegesprek ('Gaat die promotie er echt komen?') nu heb waargemaakt.

Petra Borsje en Corinda Dekker, ooit kwamen jullie me samen ophalen voor mijn vrijgezellendag, wat fijn dat jullie nu allebei als paranimf aan mijn zijde willen staan.

Lieve Petra, vanuit de collegebanken ontstond een blijvende vriendschap. We kozen een hele andere richting, maar startten allebei, jij als specialist ouderengeneeskunde, met een promotietraject. Wat leuk om de afgelopen jaren ook de promotieperikelen met elkaar te kunnen delen. Veel succes met het laatste stukje van je eigen promotie. Ons meidenclubje, met Eefke, Ingrid en Martine, is me heel dierbaar. Dankzij jouw vasthoudendheid gaan we nooit uit elkaar, zonder dat er een nieuwe datum gepland staat. Inmiddels hebben we onze 25-jarige vriendschap gevierd. Ik hoop nog veel 'meidenmomenten' met jullie te delen.

Lieve Corinda, vriendin vanaf de eerste klas van de lagere school, waar we 38 jaar geleden door de juf naast elkaar werden gezet. Al hebben we na de schoolperiode uiteenlopende wegen bewandeld, toch zijn we altijd verbonden gebleven. Ook als we elkaar door allerlei omstandigheden minder vaak zien dan dat we eigenlijk zouden wil-

len, blijft er toch altijd dat vertrouwde gevoel, kletsen we door waar we gebleven waren en kunnen we alles delen. Onze vriendschap is heel waardevol voor mij.

Dit proefschrift draag ik op aan mijn ouders. Lieve Pa en Ma, dank jullie wel voor de warme en stabiele basis die jullie mij gegeven hebben. Jullie hebben mij de mogelijkheden geboden en gestimuleerd om me steeds verder te ontwikkelen. Dank voor jullie onvoorwaardelijke liefde en steun. Fijn dat jullie er altijd voor me waren en nog steeds zijn, ik hoop dat dat nog lang zo zal blijven. Ma, dank ook voor het kritisch doornemen van de Nederlandse samenvatting.

Tot slot mijn 3 mannen. Lieve Hans, naast mijn promotietraject waren er diverse andere 'projecten' in de afgelopen jaren. Ik ben trots op wat we samen hebben bereikt. Ik wil je bedanken voor je liefde en geduld. Na ons recente koper, ga ik graag samen met je op naar zilver! Lieve Laurens en Arnoud, wat ben ik blij met jullie. Ik geniet van jullie enthousiasme, humor, fantasie, sportiviteit, knuffels, onverwachte vragen en meer. Met z'n vieren gaan we vast nog veel moois beleven!