Cortisol and Cortisone in Early Childhood in Very-Low-Birthweight Infants and Term-Born Infants

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Keywords

Cortisol · Cortisone · 11 β -hydroxysteroid dehydrogenase type 2 · Metabolic syndrome · Preterm infants

Abstract

Introduction: Besides programming of the hypothalamic-pituitary-adrenal (HPA) axis, changes in the activity of 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) could contribute to the later metabolic and cardiovascular consequences of preterm birth. **Objective:** We compared serum cortisol, cortisone, and cortisol/cortisone ratio in early childhood in very-low-birthweight (VLBW) infants and term appropriate for gestational age (AGA) born infants. **Methods:** We included 41 VLBW infants, participating in the randomized controlled Neonatal Insulin Replacement Therapy in Europe trial, and 64 term AGA-born infants. Cortisol and cortisone were measured in blood samples taken at 6 months and 2 years corrected age (VLBW children) and at 3 months and 1 and 2 years (term children). At 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 VLBW children compared to term children. In the total group of children, cortisol/cor-

tisone ratio is positively related to triglycerides at 2 years of (corrected) age. In VLBW 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 VLBW infants, lower 11 β -HSD2 activity probably contributes to the long-term metabolic and cardiovascular risks. In VLBW infants, early insulin treatment could affect programming of the HPA axis, resulting in higher cortisol and cortisone levels during early childhood.

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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-birthweight (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 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) contribute to the metabolic and cardiovascular consequences in later life [5, 6]. 11 β -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 11 β -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 11 β -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 (birthweight <1,500 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-year 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 relationship between F/E ratio and components of the metabolic syndrome at 2-year (corrected) age in the total group of children (VLBW and term AGA). 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 h 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 birthweight above the 10th percentile [10]. Standard deviation scores of birthweight 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 h after birth. In our unit, 47 VLBW infants participated in the NIRTURE trial. Five infants died and 1 child was excluded because parents refused blood sampling at the follow-up visits; 41 VLBW children were included in the present study. At 2-year corrected age, one of these 41 children was lost to follow-up. Four (10%) of the VLBW children were SGA (defined as a birthweight below the 10th percentile [10]). Thirty-nine infants received antenatal steroids 1 ($n = 13$), 2 ($n = 24$), or 3 ($n = 2$) 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 and 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 of corrected age in the VLBW infants and at 3 months and 1 and 2 years of age in the term born infants, according to the specific study protocol.

Blood samples taken at 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 for insulin resistance (HOMA-IR = [fasting insulin mU/L $\times$ fasting glucose mmol/L]/22.5) [15].

All blood samples were taken early in the afternoon after a fasting period of at least 3 h. Samples were stored at -80°C and were all analyzed at the same time. Study population and data collection also have been previously described [3, 4, 16, 17].

Table 1. Characteristics of the VLBW and term AGA children

	VLBW (<i>n</i> = 41)	Term AGA (<i>n</i> = 64)	<i>p</i> value
Sex	21 M/20 F	35 M/29 F	0.73
Gestational age, weeks	27.9±1.3	39.3±1.2	<0.001
Birthweight, g	1,059±231	3,529±393	<0.001
Birthweight 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 Caucasians, 10 Blacks, 3 Moroccans, and 2 Asians	55 Caucasians, 4 Blacks, and 5 Asians	
Highest level of parental education ^a	3 low, 18 medium, and 20 high	1 low, 19 medium, 35 high, and 9 unknown	
Breastfeeding	31/41 (76%)	45/64 (70%)	0.55
Duration of exclusive breastfeeding, months	3 (0–8)	3 (0–6)	0.21
Total duration of breastfeeding, months	5 (1–23)	4 (1–24)	0.92
Weight, g at expected date of delivery ^b	3,154±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 breastfeeding is presented as median (range). VLBW infants are compared to term AGA infants. VLBW, very low birthweight; AGA, appropriate for gestational age. ^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 birthweight (SDS) of the term AGA infants.

Assays

For measurement of serum cortisol and cortisone samples were prepared as described by Hawley et al. [18]; concentrations were assessed by the 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 coefficient of variation is 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, PA, USA). Lower limit of quantitation is 10 pmol/L; intra-assay coefficient of variation is 4% at 20 pmol/L, 3% at 500 pmol/L, and 4% at 1,500 pmol/L; inter-assay coefficient of variation is 8% at 24 pmol/L and 7% at both 780 pmol/L and 3,000 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, IL, USA) and Stata version 14 (StataCorp, College Station, TX, USA). Differences in characteristics between VLBW children and term AGA children were evaluated using in-

dependent Student's *t* test for normally distributed variables, Mann-Whitney test for not normally distributed variables, and χ^2 tests for dichotomous and categorical variables. 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 vs. girls and early insulin vs. standard care) were analyzed with linear mixed model analyses. Linear mixed model analyses were used to adjust for the dependency of the observations within the child.

Bivariate correlation analysis was performed to study the relation between F/E ratio and several components of the metabolic syndrome and between F/E ratio and growth parameters.

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.

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

Table 2. Serum cortisol, cortisone, and ratio

	VLBW (n = 41)	Term AGA (n = 64)
At 3 months of (corrected) age		
Cortisol, nmol/L		255 (135–389)
Cortisone, nmol/L		89 (65–105)
Ratio cortisol/cortisone		2.8 (1.9–3.6)
At 6 months of (corrected) age		
Cortisol, nmol/L	215 (161–295)	
Cortisone, nmol/L	65 (49–78)	
Ratio cortisol/cortisone	3.7 (2.9–4.3)	
At 1 year of (corrected) age		
Cortisol, nmol/L		245 (197–319)
Cortisone, nmol/L		58 (40–73)
Ratio cortisol/cortisone		4.3 (3.7–5.2)
At 2 years of (corrected) age		
Cortisol, nmol/L	200 (153–348)	243 (176–296)
Cortisone, nmol/L	47 (33–72)	61 (44–70)
Ratio cortisol/cortisone	4.7 (4.0–6.1)	4.1 (3.5–5.0)
Difference between the VLBW and term AGA children on average over time adjusted for gender		
Cortisol, nmol/L	0.99 ^a (0.79–1.22); <i>p</i> = 0.89	
Cortisone, nmol/L	0.88 ^a (0.75–1.03); <i>p</i> = 0.10	
Ratio cortisol/cortisone	0.62 ^b (0.06–1.19); <i>p</i> = 0.03	

Data are expressed as median and interquartile range. ^a Difference expressed as a ratio (95% confidence interval) (VLBW compared to term AGA; VLBW, lower values than term AGA). ^b Unstandardized regression coefficient obtained from a mixed-model analysis.

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

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 two 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

Descriptive information regarding metabolic syndrome components at 2 years of (corrected) age was reported earlier [16]. In the total group of children (VLBW and term AGA), F/E ratio was significantly correlated to triglycerides (*r* = 0.22; *p* = 0.036) at 2 years of (corrected) age. F/E ratio was not correlated to total cholesterol, HDL cholesterol, glucose, insulin, and HOMA-IR.

Relation of F/E Ratio to Growth Parameters

In the total group of children (VLBW and term AGA), F/E ratio was significantly correlated to weight (*r* = −0.25; *p* = 0.017) and BMI (*r* = −0.23; *p* = 0.026) at 2 years of (corrected) age. F/E ratio was not significantly correlated to length at 2 years of (corrected) age.

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 healthy subjects, F/E ratio rises during the first year of life, stays unchanged until adulthood and then increases with age. The increase of F/E ratio indicates a decrease in 11β-HSD2 activity and may contribute to the increase of blood pressure in early childhood and in elderly persons [21, 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 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. Besides these indications for increased activity

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

	Girls (<i>n</i> = 20)	Boys (<i>n</i> = 21)	Standard care (<i>n</i> = 24)	Early insulin (<i>n</i> = 17)
At 6 months of corrected age				
Cortisol, nmol/L	233 (163–328)	201 (156–253)	204 (154–264)	242 (174–318)
Cortisone, nmol/L	71 (53–83)	64 (45–75)	63 (49–76)	69 (44–82)
Ratio cortisol/cortisone	3.8 (3.0–4.3)	3.7 (2.6–4.3)	3.7 (2.8–4.1)	3.8 (3.2–4.6)
At 2 years of corrected age				
Cortisol, nmol/L	208 (136–340)	195 (179–361)	181 (133–208)	313 (202–373)
Cortisone, nmol/L	44 (31–72)	47 (37–75)	36 (31–50)	72 (50–77)
Ratio cortisol/cortisone	4.7 (3.9–6.2)	4.8 (4.2–6.0)	4.6 (3.9–5.7)	5.2 (4.0–6.2)
Difference between the groups on average over time				
Cortisol serum, nmol/L	0.99 ^a (0.78–1.36); <i>p</i> = 0.93		1.39 ^a (1.12–1.75); <i>p</i> = 0.003	
Cortisone serum, nmol/L	0.99 ^a (0.82–1.19); <i>p</i> = 0.90		1.28 ^a (1.08–1.53); <i>p</i> = 0.005	
Ratio cortisol/cortisone	0.09 ^b (–0.59 to 0.72); <i>p</i> = 0.80		0.31 ^b (–0.38 to 0.99); <i>p</i> = 0.38	

Data are expressed as median and interquartile range. VLBW, very low birthweight. ^a Difference expressed as a ratio (95% confidence interval) (boys vs. girls and early insulin vs. standard care). ^b Unstandardized regression coefficient obtained from a mixed-model analysis.

of the HPA axis based on basal cortisol levels, studies investigating cortisol reactivity to stress suggest lower HPA axis response to psychosocial stress in VLBW young adults [30] and to immunization pain in preterm-born boys at 4 months of corrected age [31], both compared to term-born controls. Cumulative cortisol levels in hair at 7 years of age were found to be lower in preterm-born children compared to term-born controls [32]. Taken together, these studies suggest dysregulation in cortisol levels in preterm-born children compared to term-born controls.

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 of 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 of corrected age suggests the contribution of programming of the HPA axis already in early childhood [4]. In accordance with this, Grunau et al. [33] 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 of 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 been investigated earlier in early childhood in VLBW infants.

Houang et al. [5] studied F/E ratio in IUGR-born children without catch-up growth at 1.1–13.5 years of age. They showed that 20% of these children had high F/E ratios compared to controls, suggesting partial 11 β -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-IR [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. Higher F/E ratio could not only be caused by decreased 11 β -HSD2 activity but also by increased activity of 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1), which converts cortisone into cortisol and is mainly active in the liver [21]. However, in rats, maternal malnutrition during pregnancy results in raised systolic blood pressure and markedly reduced levels of 11 β -HSD2 expression in the kidney and adrenal of the offspring; levels of 11 β -HSD1 expression remain unchanged. This indicates that expression of 11 β -HSD2, and not 11 β -HSD1, is involved in long-term programming of glucocorticoid hormone action [34].

The results of our present study therefore suggest that in VLBW infants, low 11 β -HSD2 activity could 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 [35, 36].

In SGA born children, F/E ratio is positively correlated with total and LDL cholesterol levels [5, 6]. In our total group of children (VLBW and term AGA), F/E ratio at 2 years of (corrected) age was positively correlated with triglycerides. This could indicate that F/E ratio, and hence 11 β -HSD2 activity, is associated with metabolic risks.

Decreased 11 β -HSD2 activity is also associated with reduced (catch-up) growth in childhood, probably due to glucocorticoid imbalance; in SGA-born children, F/E ratio is negatively correlated with height [5, 6]. We also found an inverse relation between F/E ratio and growth: in our total group of children, there was a negative correlation between F/E ratio and weight and between F/E ratio and BMI.

We earlier showed that VLBW children treated with insulin in the first postnatal week have higher serum cortisol levels at 2 years of 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 11 β -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, 33]. Further studies are necessary to provide more clarity about cortisol, cortisone, and the F/E ratio in VLBW children. 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 difference in F/E ratio suggested by the results of the present study and to find out whether a higher F/E ratio persists in older children. Future studies should also include measurement of growth

parameters and of several metabolic syndrome components, including blood pressure, at several time points in order to further investigate the relationship between F/E ratio and metabolic parameters in children and between F/E ratio and growth.

In conclusion, VLBW infants have higher F/E ratio during early childhood compared to term-born children, suggesting lower 11 β -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.

Statement of Ethics

The VLBW infants were part of the randomized controlled Neonatal Insulin Replacement Therapy in Europe (NIRTURE) trial (EudraCT number 2004-002170-34). Written informed consent was obtained from both parents. Approval from the ethics committee of the VU University Medical Center was obtained (No. 2004/121).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

M.d.J.: conception and design, data collection, analysis and interpretation of data, writing original draft, and review and editing of the manuscript. A.C.: data collection and review and editing of the manuscript. J.T.: statistical analysis and review and editing of the manuscript. M.v.W.: conception and design, analysis and interpretation of data, review and editing of the manuscript, and supervision.

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