Outcomes 8 years after preterm birth

Ruys, C.A.

2019

document version
Publisher's PDF, also known as Version of record

Chapter 6

Birth weight and postnatal growth in preterm-born children are associated with cortisol in early infancy, but not at age 8 years

Charlotte A. Ruys,* Bibian van der Voorn,* Harrie N. Lafeber, Monique van de Lagemaat, Joost Rotteveel, Martijn J.J. Finken

* Authors contributed equally to this manuscript

Psychoneuroendocrinology. 2017;82:75-82

ABSTRACT

Background Preterm birth has been associated with altered hypothalamic-pituitary-adrenal (HPA-) axis activity as well as cardiometabolic diseases and neurodevelopmental impairments later in life. We assessed cortisol from term age to age 8 years in children born preterm, to explore the development of HPA-axis activity in association with intrauterine and early-postnatal growth until 6 months corrected age (CA).

Methods In 152 children born at a gestational age ≤ 32 weeks and/or with a birth weight ≤ 1500 g, random serum cortisol was assessed at term age (n = 150), 3 months (n = 145) and 6 months CA (n = 144), and age 8 years (n = 59). Salivary cortisol was assessed at age 8 years (n = 75): prior to bedtime, at awakening, 15 min after awakening, and before lunch. Cortisol was analyzed in association with birth weight-standard deviation score (SDS), being born small for gestational age (SGA), and combinations of intrauterine and postnatal growth: appropriate for gestational age (AGA) with or without growth restriction (AGA GR+ or AGA GR-) at 6 months CA, and SGA with or without catch-up growth (SGA CUG+ or SGA CUG-) at 6 months CA. Cross-sectional associations at all time points were analyzed using linear regression, and longitudinal associations were analyzed using generalized estimating equations.

Results Longitudinally, birth weight-SDS was associated with cortisol (β [95% CI]): lower cortisol over time was seen in infants with a birth weight ≤ -2 SDS (-50.69 [-94.27; -7.11], P = 0.02), infants born SGA (-29.70 [-60.58; 1.19], P = 0.06), AGA GR+ infants (-55.10 [-106.02; -4.17], P = 0.03) and SGA CUG- infants (-61.91 [-104.73; -19.10], P = 0.01). In cross-sectional analyses at age 8 years, no associations were found between either serum or salivary cortisol and birth weight-SDS, SGA-status, or growth from birth to 6 months CA.

Conclusion In children born preterm, poor intrauterine and postnatal growth were associated with lower cortisol in early infancy, but not at age 8 years. Even though HPA-axis activity no longer differed between groups at age 8 years, or differences could not be confirmed due to attrition, it is unknown whether the differences found in early infancy could attribute to increased health risks later in life.

INTRODUCTION

In infants born very preterm, i.e., born at a gestational age ≤ 32 weeks, the hypothalamic-pituitary-adrenal (HPA-) axis is not yet fully maturated. Relative adrenal insufficiency is common in this group during the first weeks of life and is characterized by relatively low basal and stress-induced cortisol levels, and increased risks of hypotension, hypoglycemia and bronchopulmonary dysplasia.[1-4] This is partly attributable to the sudden disruption of the maturation of the fetal HPA-axis, which in full-term pregnancies is stimulated in the third trimester by an increase in the secretion of placental corticotropin-releasing hormone (CRH) and alterations in the expression of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) by the placenta.[1] Conversely, there is some evidence suggesting that HPA-axis activity is upregulated years after preterm birth,[2] which might contribute to the association between prematurity and long-term sequelae like cardiometabolic diseases and neurodevelopmental impairments.

Little is known about the impact of intrauterine and early-postnatal growth patterns on these associations in preterm infants. Intrauterine growth restriction is accompanied by a reduced expression and activity of the placental barrier enzyme 11β-HSD2, which converts cortisol to inert cortisone.[5] The subsequent fetal overexposure to maternal cortisol has been suggested to permanently alter HPA-axis settings,[6] initially by suppressing the axis, followed by increased activity later in life. This is strengthened by animal studies suggesting that the presence of abundant glucocorticoids in-utero could result in a reduced expression of glucocorticoid receptors in tissues, and thereby, a compensatory upregulation of HPA-axis activity.[7-9]

Moreover, animal studies suggest that fetal growth restriction as well as early-postnatal growth restriction may predispose to cardiometabolic diseases later in life. Also, rapid postnatal growth after being born with a low birth weight has been associated with cardiometabolic disease risk, and alterations in HPA-axis functioning have been suggested to explain these associations.[9,10]

In term-born subjects, there are few studies that have explored whether the HPA-axis could explain part of the association between low birth weight and cardiometabolic disease.[11,12] In preterm infants, rapid early-postnatal and childhood growth have been associated with risks of cardiometabolic diseases,[13,14] but their relation with HPA-axis activity throughout the lifespan has never been described.

To study the development of HPA-axis activity after preterm birth in association with intrauterine and early-postnatal growth, we assessed cortisol levels of infants who were born very preterm and/or with a very low birth weight (≤ 1500 g), from term age until the age of 8 years.

METHODS

All subjects were originally included in a nutritional RCT ('Study Towards the Effects of Postdischarge nutrition' (STEP-1)) that compared the effects of postdischarge formula, term formula, and human milk on growth and body composition of very preterm (gestational age ≤ 32 weeks) and/or very low birth weight (≤ 1500 g) infants, as described previously.[15] Exclusion criteria were congenital malformations or other conditions known to affect growth or body composition. At term age, infants fed formula were randomized to receive either a protein- and mineral-enriched postdischarge formula or term formula between term age and 6 months corrected age (CA). Corrected age is the age of the preterm-born child calculated from the term date (i.e., 40 weeks gestation), and not from birth. This correction is usually maintained until the CA of 24 months.

At the age of 8 years, parents from the STEP-1 study participants were asked to participate in the follow-up study, STEP-2. Exclusion criteria were incomplete follow-up, severe physical impairment or other conditions known to affect growth or body composition.

Data collection

For STEP-1, the following data were extracted from medical records: birth weight, birth length, gestational age, and gender. The neonatal therapeutic intervention scoring system (NTISS), an indicator for neonatal illness severity and mortality risk,[16] was calculated, and parents were asked to report their ethnicity, which was categorized as Caucasian or non-Caucasian. At term age, 3 months and 6 months CA, weight was measured with a digital scale to the nearest 1.0 g, and length with a length board to the nearest 0.1 cm. Standard deviation scores (SDS's), which quantify the deviation from a reference population, were calculated for all auxological parameters. At birth and at term age, this was done by the use of neonatal anthropometric charts, adjusting for sex and gestational age.[17] At 3 and 6 months CA, this was done by the use of postnatal growth curves, adjusting for sex and CA.[18] At term age, 3 months and 6 months CA, fasting venous blood samples were collected. Mean fasting duration was recorded as the interval between blood sampling and the last feed before blood sampling. Mean fasting duration was 3.4 ± 0.7 h at term age, 3.6 ± 0.7 h at 3 months CA, and 3.5 ± 0.7 h at 6 months CA.

We used the following definitions:[19]

1. Appropriate for gestational age (AGA): birth weight and birth length > -2 SDS.
2. Growth restriction (AGA GR+): weight and/or length ≤ -2 SDS at 6 months CA, after being born AGA.

3. Small for gestational age (SGA): birth weight and/or birth length ≤ -2 SDS.
4. Catch-up growth (SGA CUG+): weight and length > -2 SDS at 6 months CA, after being born SGA.

For STEP-2, children aged 8 years visited the outpatient clinic in the morning. Venous blood samples were obtained after an overnight fast and anthropometric measurements were performed. Weight was measured to the nearest 0.05 kg with an electronic scale (Seca) and standing height was measured to the nearest 0.1 cm using a digital stadiometer (DGI 250D, De Grood Metaaltechniek, Nijmegen, The Netherlands) and expressed as SDS based on Dutch reference data adjusted for sex and age.[18] Salivary samples for cortisol measurement were collected at 4 moments: prior to bedtime at the evening before the study visit, immediately after awakening at the morning of the study visit, 15 min after awakening, and before lunch during the study visit. Participants were instructed to refrain from eating and drinking at least 30 min before sampling.

Parents were asked to report their education level, which was categorized as neither, one, or both parent(s) having finished higher education.

The study protocols were approved by the ethics committee of VU University Medical Center, Amsterdam. All parents of subjects gave written informed consent.

Laboratory parameters

Serum was stored at -80°C and thawed only once just before the analyses.

In STEP-1, total serum cortisol (nmol/L) was measured using 2 different methods as the assay changed during the course of the study. In 90 infants, total serum cortisol at term age, 3 months and 6 months CA was measured using a competitive immunoassay (Advantage, Nichols Institute Diagnostics, San Juan Capistrano, USA) with an intra-assay coefficient of variation (CV) of 3%, 3% and 4% (at levels of 100, 500 and > 600 nmol/L, respectively), an inter-assay CV of 8%, 7% and 6% (at levels of 140, 400 and 850 nmol/L, respectively), and a lower limit of quantitation (LLOQ) of 30 nmol/L. In 62 infants, total serum cortisol at term age, 3 months and 6 months CA age was measured using a competitive immunoassay (DPC, Los Angeles, USA) with an inter-assay CV of 8%, 7% and 6% (at levels of 150, 400 and 900 nmol/L, respectively).

In STEP-2, total serum cortisol was measured using a competitive immunoassay (Luminescence Advia Centaur, Siemens Medical Solutions Diagnostics, USA) with an intra-assay CV of 3% (at a level of 700 nmol/L), an inter-assay CV of 7%, 6% and 6% (at levels of 80, 300 and 1000 nmol/L, respectively), and a LLOQ of 30 nmol/L.

Salivary samples were stored at -80°C and thawed just before analyses. Free cortisol in saliva was measured using a competitive immunoassay (Luminescence Architect, Abbott Laboratories, Diagnostics Division Abbott Park, Illinois, USA) with an intra-assay CV of 9% (at a level of 5 nmol/L), an inter-assay CV of 11% (at a level of 7 nmol/L), and a LLOQ of 1 nmol/L.

All laboratory tests were performed by the Endocrine Laboratory of VU University Medical Center, Amsterdam, The Netherlands.

Data analysis

To assess the effect of attrition at follow-up, baseline characteristics of participants, nonparticipants and excluded subjects were compared using one-way ANOVA, Chi-square or Kruskal-Wallis tests, with STEP-2 participants as reference group (**Table 6.1**). Since cortisol is associated with body mass index (BMI), we compared BMI at age 8 years between the groups. No significant differences were found between SGA and AGA groups ($P = 0.560$), and we therefore decided not to adjust our cross-sectional analyses at age 8 years for BMI.

Serum cortisol

To test whether groups differed at any of the time points, cross-sectional, univariable, linear regression analyses were performed with either cortisol at term age, 3 months or 6 months CA, or 8 years as dependent factor.

Subsequently, generalized estimating equations (GEEs) were used for longitudinal analysis of cortisol, i.e., the assessment of differences between groups, adjusted for intra-individual variation over time. We assumed that attrition at age 8 years resulted in 'missings completely at random' and therefore used all available data of participants of the original RCT ($n = 152$) without exclusion of dropouts, while accounting for 'missings completely at random' by use of GEE. GEE is designed for the handling of missing data, provided that missingness is completely at random.[20,21] In addition, GEE adjusts for grouped samples, collected from the same subject at different times, by using a correlation structure. For our data analyses, we chose an exchangeable correlation structure, in which one average within-subject correlation between samples over time is assumed. Stepwise GEEs were performed with cortisol over time (at term age, 3 months CA, 6 months CA, and 8 years) as dependent, continuous factor. First, the association between birth weight-SDS and cortisol was investigated using univariable regression, with birth weight-SDS as independent, continuous factor. Second, the association between birth weight ≤ -2 SDS and cortisol

Table 6.1. Baseline characteristics of all subjects, and STEP-2 participants, STEP-2 nonparticipants and excluded subjects

		All subjects	STEP-2 Participants	STEP-2 Nonparticipants	STEP-2 Excluded subjects	*P* value[a]
n		152	79	52	21	
Perinatal characteristics						
Male		78 (51)	40 (51)	26 (50)	12 (57)	NS
Gestational age	wks.	30 [30; 31]	31 [29; 32]	30 [30; 31]	31 [30; 32]	NS
	range	25; 33				
Birth weight	g	1339 ± 294	1314 ± 304	1350 ± 290	1404 ± 265	NS
	range	710; 2065				
	SDS	-0.3 ± 1.0	-0.4 ± 1.0	-0.3 ± 0.9	-0.2 ± 1.2	NS
	range	-2.8; 1.9				
Birth length	cm	38 ± 3.0	38 ± 3.1	38 ± 3.0	38 ± 2.9	NS
	SDS	-0.9 ± 1.2	-0.8 ± 1.3	-1.1 ± 1.1	1.0 ± 1.3	NS
	range	-3.9; 2.0				
SGA		35 (23)	17 (22)	13 (28)	5 (24)	NS
Birth head circumference	cm	27.7 ± 1.9	27.6 ± 1.9	27.7 ± 1.9	28.0 ± 1.8	NS
	SDS	-0.2 ± 1.1	-0.2 ± 1.1	-0.3 ± 1.0	-0.21 ± 1.3	NS
NTISS		21.4 ± 7.6	21.9 ± 7.8	21.4 ± 7.1	19.8 ± 8.0	NS
Weight 6 months CA	kg	7.3 ± 1.1	7.2 ± 1.0	7.4 ± 1.0	7.6 ± 1.4	NS
	SDS	-0.4 ± 1.2	-0.5 ± 1.2	-0.3 ± 1.2	-0.3 ± 1.7	NS
Length 6 months CA	cm	66.4 ± 2.9	66.4 ± 2.8	66.5 ± 2.9	66.4 ± 3.4	NS
	SDS	-0.3 ± 1.1	-0.4 ± 1.1	-0.3 ± 1.1	-0.5 ± 1.4	NS
Demographics						
Parents who finished higher education	neither	77 (52)	41 (53)	23 (45)	13 (62)	NS
	one	32 (22)	15 (19)	13 (26)	4 (21)	
	both	39 (26)	22 (28)	15 (29)	2 (11)	
≥ 1 parent non-Caucasian		43 (28)	17 (22)	13 (25)	13 (62)	< 0.01

Data are expressed as mean ± SD, median [IQR], or *n* (%). Abbreviations: CA, corrected age; NS, non-significant; NTISS, neonatal therapeutic intervention scoring system; SDS, standard deviation score; SGA, small for gestational age (birth weight and/or birth length ≤ -2 SDS).

[a] STEP-2 participants, STEP-2 nonparticipants and excluded subjects were compared using one-way ANOVA, Chi-square or Kruskal-Wallis tests, as appropriate.

was investigated, with birth weight-SDS as independent, dichotomous factor. Third, the association between SGA-status and cortisol was investigated, with SGA-status as independent, dichotomous factor. Fourth, infants were classified into 4 groups according to birth weight-SDS and growth from birth to 6 months CA (see also Methods section): AGA GR- (1), AGA GR+ (2), SGA CUG- (3) and SGA CUG+ (4). Subsequently, the

influence of growth pattern on cortisol was investigated, with the groups as independent, categorical factor and AGA GR- as reference category. Fifth, in order to test the stability of our data, all analyses were repeated, adjusted for perinatal or other characteristics that were significantly different between AGA and SGA subjects, one by one (**Table 6.2**).

Table 6.2. Perinatal and study characteristics that could potentially confound the research question

	AGA	SGA	*P* value[a]
n	109	35	
PIH/Preeclampsia/HELLP	36 (33)	21 (60)	< 0.01
Antenatal glucocorticoid treatment	57 (52)	24 (69)	< 0.01
Male	49 (45)	25 (71)	< 0.01
Gestational age (wks.)	30 [29; 31]	31 [30; 32]	< 0.01
STEP-1 human milk group	37 (34)	11 (31)	NS
NTISS score	21.4 ± 7.8	21.6 ± 6.9	NS
Caucasian ethnicity	78 (72)	24 (69)	NS
Time of collection serum samples (24h)[b]	13:27 ± 2:05	13:29 ± 2:09	NS

Data are expressed as mean ± SD, median [IQR], or *n* (%). Abbreviations: AGA, appropriate for gestational age (birth weight and birth length > -2 SDS); HELLP, Hemolysis Elevated Liver enzymes and Low Platelets; NS, non-significant; NTISS, neonatal therapeutic intervention scoring system; PIH, pregnancy induced hypertension; SGA, small for gestational age (birth weight and/or birth length ≤ -2 SDS).
[a] Groups were compared using independent t-test, Fisher's exact test, or Mann-Whitney U test as appropriate.
[b] Mean time of collection of all sampling moments: term age, 3 months or 6 months corrected age and age 8 years.

Salivary cortisol at age 8 years

Data were examined for outliers. Levels at awakening that were 10 times higher than 15 min after awakening, and levels in the evening that were 10 times higher than the cohort mean, were excluded.

The cortisol awakening response (CAR) was calculated as the difference between levels at awakening and 15 min after awakening. We calculated the area under the curve increase (AUC_i; with reference to the lowest individual cortisol level) of all samples according to the formula by Pruessner,[22] and a time-weighted AUC_i (divided by the total time of collection, AUC_i/h). Analyses of salivary cortisol parameters in association with birth weight-SDS, SGA-status and growth from birth to 6 months CA were performed with univariable linear regression analysis.

Statistical significance was defined as a *P* value of < 0.05. Statistical analyses were performed with IBM SPSS Statistics version 22.

RESULTS

One hundred and fifty-two subjects were included in STEP-1. Over time, 13 children were lost to follow-up, 21 children were excluded, and 39 children refused to participate in STEP-2, resulting in 79 subjects that could be included at age 8 years in STEP-2 (**Figure 6.1**). No differences were found in baseline characteristics of STEP-2 participants vs. nonparticipants and excluded subjects ($P > 0.3$), except for parental ethnicity which was more often "non-Caucasian" in the excluded group compared to participants and nonparticipants (**Table 6.1**).

AGA and SGA subjects differed in gestational age, sex distribution, preeclampsia/Hemolysis Elevated Liver enzymes and Low Platelets (HELLP) syndrome and exposure to antenatal glucocorticoid treatment (**Table 6.2**). We considered these variables as possible confounders and therefore tested their influence on the longitudinal analyses.

Cross sectional analyses

Birth weight-SDS was positively associated with cortisol at 3 months and 6 months CA, β [95% CI]: 32.78 [8.74; 56.81], $P = 0.01$ and 20.90 [-0.35; 42.14], $P = 0.05$ respectively, and birth weight ≤ -2 SDS was associated with lower cortisol compared to birth weight > -2 SDS at 6 months CA (-91.60 [-172.86; -10.35], $P = 0.03$).

At 3 months CA, a non-significant association between cortisol and SGA-status was found (-49.83 [-105.85; 6.19], $P = 0.08$) (**Figure 6.2A** and **Table 6.3**).

At term age, cortisol was lower in SGA CUG- compared to AGA GR- infants (-115.69 [-212.06; -19.31], $P = 0.02$). At 3 months CA, a non-significant association between cortisol and SGA CUG+ compared to AGA GR- was found (-62.40 [-133.03; 8.23], $P = 0.08$), and at 6 months CA, a non-significant association between cortisol and SGA CUG- compared to AGA GR- was found (-64.08 [-134.65; 6.49], $P = 0.08$) (**Figure 6.2B** and **Table 6.3**).

Longitudinal analyses (GEE)

The results of the longitudinal analyses are presented in **Table 6.4**.

A birth weight < 0 SDS was associated with lower cortisol as compared to a birth weight ≥ 0 SDS (-17.33 [-30.96; -3.69], $P = 0.01$). A birth weight ≤ -2 SDS was associated with a lower cortisol over time, compared to a birth weight > -2 SDS (-50.69 [-94.27; -7.11], $P = 0.02$). Being born SGA was associated with lower cortisol over time, as compared to being born AGA (-29.70 [-60.58; 1.19], $P = 0.05$).

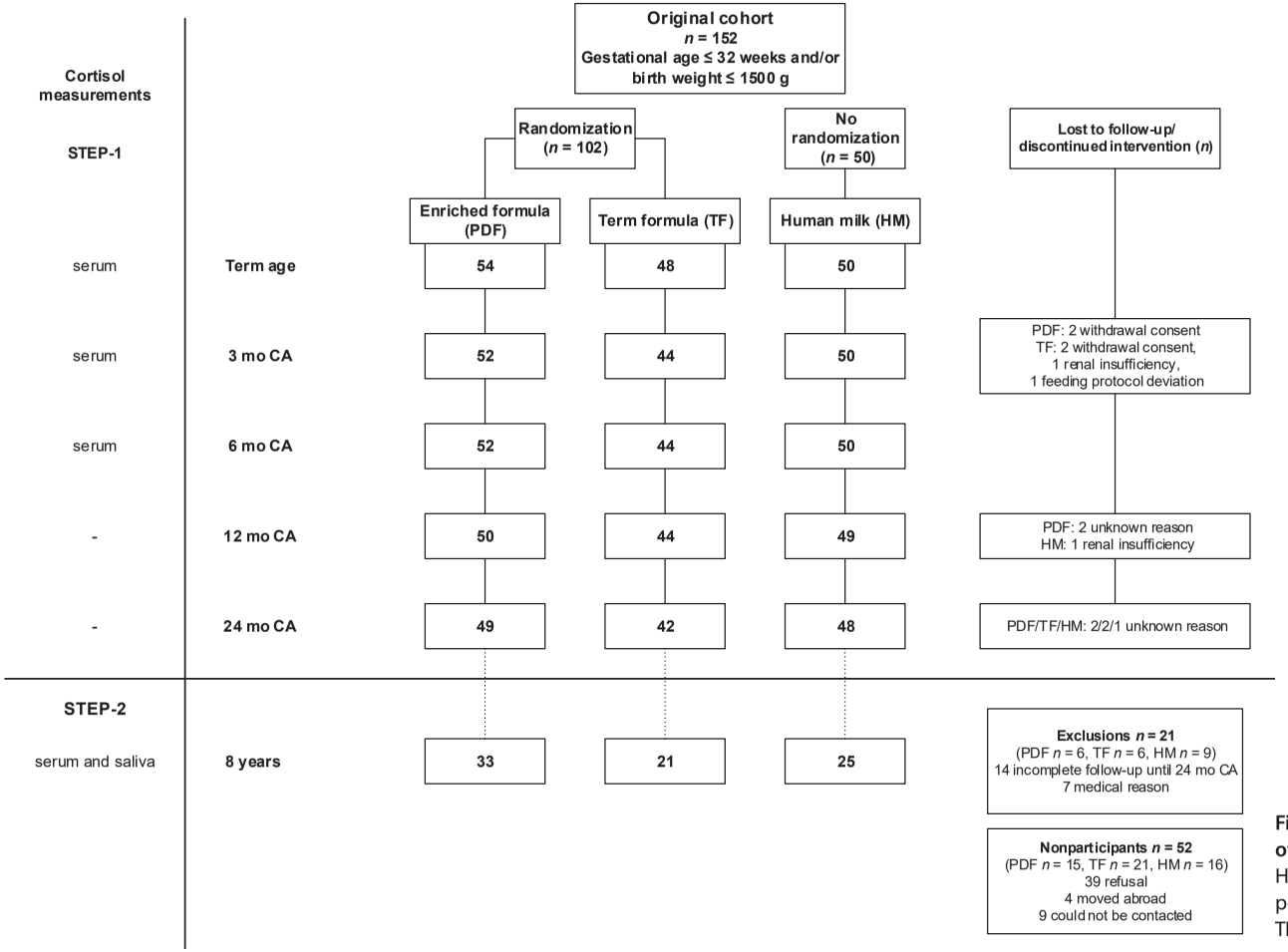

Figure 6.1. Flowchart of STEP-1 and -2.
HM, human milk; PDF, postdischarge formula; TF, term formula.

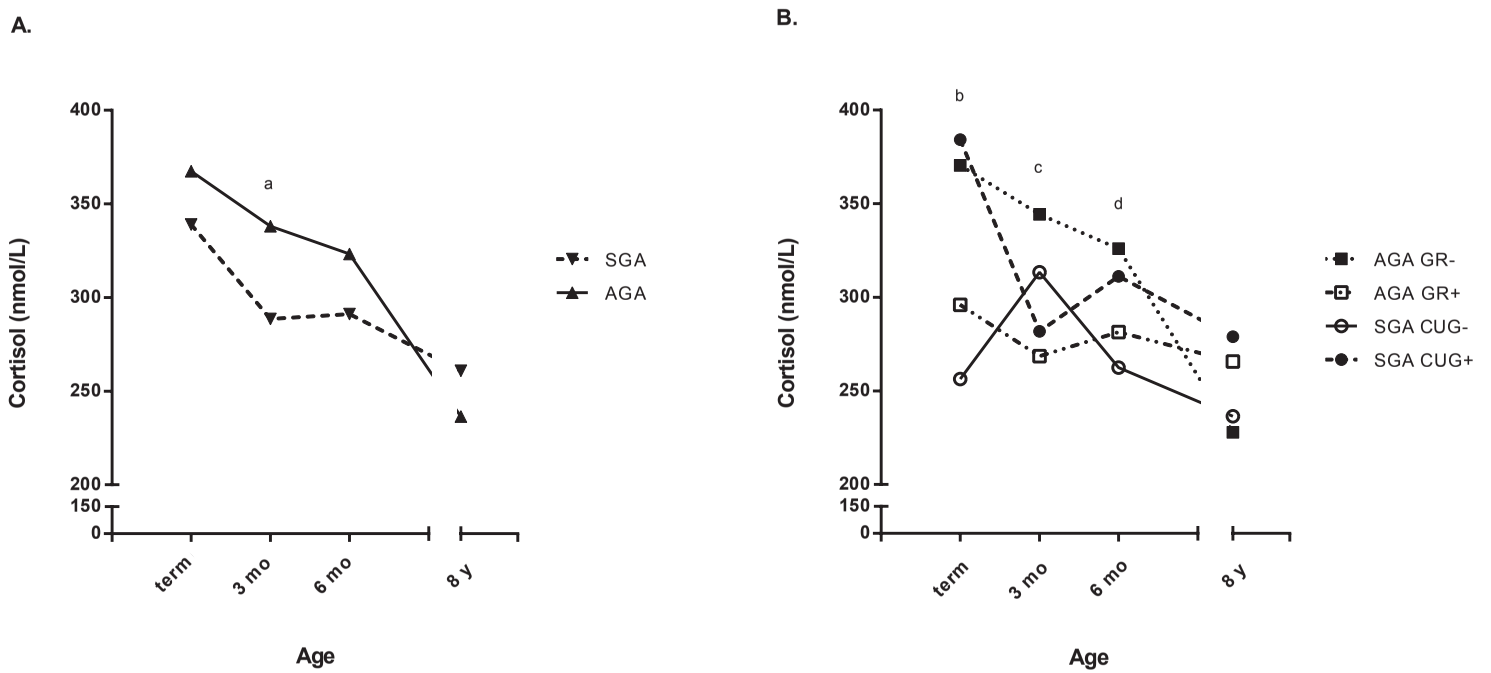

Figure 6.2. Serum cortisol (means): SGA vs. AGA infants (A), growth birth – 6 months corrected age (AGA GR-, AGA GR+, SGA CUG-, and SGA CUG+) (B).
AGA GR+/GR-, appropriate for gestational age with or without growth restriction at 6 months corrected age; SGA CUG+/CUG-, small for gestational age with or without catch-up growth at 6 months corrected age.
[a] SGA vs. AGA $P = 0.08$; [b] SGA CUG- vs. AGA GR- $P = 0.02$; [c] SGA CUG+ vs. AGA GR- $P = 0.08$; [d] SGA CUG- vs. AGA GR- $P = 0.08$. All P values represent unadjusted cross-sectional analyses.

Table 6.3. Serum cortisol (nmol/L), mean ± SD at every sampling moment

		n	Term age	*n*	3 months CA	*n*	6 months CA	*n*	8 years
Birth weight-SDS, continuous		150	367.3 ± 175.4	145	328.1 ± 148.9	144	319.8 ± 127.1	59	241.2 ± 113.5
SGA	AGA	108	367.3 ± 175.6	103	337.7 ± 143.1[a]	103	323.0 ± 126.1	43	233.7 ± 114.8
	SGA	35	339.0 ± 166.2	34	287.9 ± 143.6	34	291.1 ± 120.5	14	265.2 ± 117.5
Early postnatal growth	AGA GR-	95	372.0 ± 179.1	95	343.6 ± 146.2	95	326.5 ± 129.5	38	229.2 ± 106.8
	AGA GR+	8	296.0 ± 117.6	8	268.5 ± 73.8	8	268.7 ± 64.3	5	267.6 ± 177.1
	SGA CUG-	14	256.3 ± 157.3[b]	14	313.4 ± 110.5	14	262.4 ± 119.8[c]	6	236.7 ± 84.8
	SGA CUG+	20	384.2 ± 148.2	19	281.2 ± 159.8[d]	20	311.2 ± 119.8	8	286.6 ± 138.8

AGA, appropriate for gestational age (birth weight and birth length > -2 SDS); CA, corrected age; CUG, catch-up growth; GR, growth restriction; SGA, small for gestational age (birth weight and/or birth length ≤ -2 SDS).

[a] SGA vs. AGA $P = 0.08$, [b] SGA CUG- vs. AGA GR- $P = 0.02$, [c] SGA CUG- vs. AGA GR- $P = 0.08$, [d] SGA CUG+ vs. AGA GR- $P = 0.08$.

All *P* values represent unadjusted cross-sectional analyses.

Table 6.4. Longitudinal associations between birth weight-SDS, SGA-status, growth from birth to 6 months corrected age, and serum cortisol

				β (95% CI)	*P* value
Birth weight-SDS, continuous			152	17.33 (3.69; 30.96)	0.01
Birth weight-SDS, dichotomous	≤ -2 SDS	vs. > -2 SDS	11 vs. 141	-50.69 (-94.27; -7.11)	0.02
SGA	SGA	vs. AGA	35 vs. 109	-29.70 (-60.58; 1.19)	0.06
Early postnatal growth	AGA GR+	vs. AGA GR-	8 vs. 96	-55.10 (-106.02; -4.17)	0.03
	SGA CUG-	vs. AGA GR-	14 vs. 96	-61.91 (-104.73; -19.10)	0.01
	SGA CUG+	vs. AGA GR-	20 vs. 96	-13.26 (-49.62; 23.10)	0.48

Abbreviations: AGA, appropriate for gestational age (birth weight and birth length > -2 SDS); CI, confidence interval; CUG, catch-up growth; GR, growth restriction; SDS, standard deviation score; SGA, small for gestational age (birth weight and/or birth length ≤ -2 SDS).
All *P* values represent unadjusted longitudinal analyses.

At 6 months CA, AGA GR+ and SGA CUG - infants had lower cortisol over time compared to AGA GR- infants (-55.10 [-106.02; -4.17], $P = 0.03$, and -61.91 [-104.73; -19.10], $P = 0.01$, respectively). No differences in cortisol over time were found between SGA CUG+ and AGA GR- infants.

After adjustment for confounders, similar results were found, with the exception of pregnancy induced hypertensive diseases, which resulted in a loss of significance (data not shown). After exclusion of visually impaired STEP-1 subjects ($n = 2$), similar results were found (data not shown).

Salivary cortisol

Table 6.5 shows salivary cortisol data at age 8 years; mean per sampling moment, CAR, AUC_i, AUC_i/h, and maximum level. Two outliers with levels at awakening that were 10 times higher than 15 min after awakening, or levels in the evening that were 10 times higher than the cohort mean, were excluded from the analysis. Linear regression analyses showed no association between birth weight SDS, SGA-status or growth from birth to 6 months CA, and either of the salivary cortisol parameters (data not shown).

Table 6.5. Salivary cortisol (nmol/L), mean at every sampling moment and CAR, AUC_i and maximal level.

		n	Bedtime	Awakening	+ 15 min	Lunch	CAR	AUC_i	AUC_i/h	Max.
Birth weight-SDS , continuous		75	1.0 ± 0.1	4.9 ± 2.9	6.5 ± 3.2	2.3 ± 1.8	1.5 ± 2.8	38.2 ± 22.6	2.5 ± 1.5	6.9 ± 3.1
SGA	AGA	57	1.0 ± 0.2	4.9 ± 3.0	6.6 ± 3.4	2.3 ± 1.9	1.7 ± 2.9	38.8 ± 24.2	2.5 ± 1.5	7.1 ± 3.3
	SGA	15	1.0 ± 0.0	4.6 ± 2.2	6.0 ± 2.6	2.1 ± 1.1	1.3 ± 2.4	34.7 ± 16.1	2.2 ± 1.1	6.3 ± 2.4
Early postnatal growth	AGA GR-	51	1.0 ± 0.2	5.0 ± 3.1	6.4 ± 3.5	2.3 ± 2.0	1.4 ± 2.7	38.5 ± 25.4	3.4 ± 1.6	7.0 ± 3.4
	AGA GR+	6	1.0 ± 0.0	4.1 ± 1.9	7.9 ± 2.7	2.3 ±1.5	3.8 ± 3.7	41.6 ± 6.5	2.6 ± 0.3	8.1 ± 2.5
	SGA CUG-	5	1.0 ± 0.0	3.9 ± 1.5	6.0 ± 1.7	2.1 ± 1.0	2.0 ± 2.0	30.2 ± 11.4	1.1 ± 0.8	6.0 ± 1.7
	SGA CUG+	10	1.0 ± 0.0	4.9 ± 2.5	6.0 ± 3.0	2.1 ± 1.2	0.9 ± 2.7	37.5 ± 18.7	2.4 ± 1.2	6.6 ± 2.9

Data are expressed as mean ± SD. Abbreviations: AGA, appropriate for gestational age (birth weight and birth length > -2 SDS); AUC_i (/h), area under the curve with respect to the increase (per hour); CAR, cortisol awakening response; CUG, catch-up growth; GR, growth restriction; max, maximal salivary cortisol level of the 4 sampling moments; SGA, small for gestational age (birth weight and/or birth length ≤ -2 SDS).

DISCUSSION

We showed that differences in growth between birth and 6 months CA are related to the pattern of serum cortisol decline during infancy. In our longitudinal analyses, the growth-restricted groups (i.e., low birth weight, SGA, AGA with GR and SGA without CUG) all had a lower cortisol over time, compared to non-growth-restricted infants (AGA infants without GR). These differences were not explained by gestational age, antenatal glucocorticoid treatment or sex. Not surprisingly, statistical correction for pregnancy induced hypertensive disorders reduced the strength of our associations.[23,24] In cross-sectional analyses at age 8 years, the differences between the growth-restricted and non-growth-restricted groups were no longer present. In addition, salivary cortisol at age 8 years was not different between these groups at any sampling moment throughout the day.

In the first weeks of life, the HPA-axis of preterm newborns is still immature. Among the impairments are insensitivity of the pituitary gland to synthetic CRH, decreased 11β-hydroxylase activity in the adrenal cortex, and a cortisol-cortisone shuttle favoring cortisone.[2] In animal studies, adverse events occurring in early life have been associated with permanent alterations in HPA-axis activity.[7] In line with our results, in previous studies among infants of whom the majority were born at term, a blunted cortisol response to painful procedures was found in those born SGA.[25,26] In contrast, from childhood onwards, lower birth weight has been associated with increases in glucocorticoid metabolite excretion,[27] basal cortisol[28] and the cortisol response to psychosocial stresss.[29] Preterm infants were found to exhibit altered responses to different kinds of stressors, as compared to their term counterparts.[30,31] Furthermore, prematurity has been associated with a lower cortisol response, in spite of a higher pretest cortisol level, during a psychosocial stress test.[30,32,33] These findings suggest that the HPA-axis is hypoactive after being born SGA and/or preterm, and becomes hyperactive with age, although not indisputable. Similar shifts were observed in extremely preterm infants (gestational age < 29 wks) compared to very preterm (gestational age 29–32 wks) and term infants.[34] Our study suggests that these longitudinal patterns in HPA-axis activity of preterm infants are augmented by poor intrauterine and early-postnatal growth, although our follow-up might have been too short to demonstrate a subsequent increase in HPA-axis activity.

We found no differences in salivary cortisol parameters at age 8 years, which included diurnal rhythmicity and CAR, between growth-restricted and non-growth-restricted subjects. At school age, a lower CAR has been described in preterm-born children compared to term-born control subjects.[35] Studies regarding the diurnal rhythm linked preterm birth with cortisol levels that were either higher at bedtime[36] or throughout the day[37]. Since we

did not include a term control group, the results of these studies cannot be compared to ours. Moreover, we collected only 2 salivary samples post-awakening, and may therefore have missed the peak cortisol concentration. In addition, a single collection day may not be sufficient to demonstrate differences at age 8 years.[38]

It is increasingly recognized that preterm birth constitutes a major risk factor for cardiometabolic disease.[39] However, in studies providing long-term follow-up of preterm populations, no differences in insulin resistance or blood pressure were demonstrated between subjects with and without intrauterine growth restriction.[40,41] Possibly, the subjects within these cohorts were too young to demonstrate such differences. This may also partly explain the lack of association in our sample at age 8 years. Life-long follow-up of preterm populations is warranted, since it is conceivable that alterations in HPA-axis activity in preterm infants during early life are involved in pathways leading to cardiometabolic disease and neurodevelopmental impairments.

Strengths and limitations

The main strength of this study is the use of a well-described birth cohort, in which extensive information on early growth was available. In addition, serial measurement of serum cortisol in early infancy as well as in childhood, and measurement of salivary cortisol at age 8 years were performed.

There are several limitations. First, this study was a post hoc analysis of a nutritional RCT, in which a term born control group was not included. Second, attrition at age 8 years limited our sample size. We therefore carefully assessed the possibility of attrition bias by comparing participants with nonparticipants and excluded subjects. Since there were no differences, analyzing with GEE gave us the opportunity to use all available data in longitudinal analyses, while accounting for missing data.[20] With this approach, we followed the suggested reporting requirements for addressing attrition as described by Fewtrell.[21] Third, subgroup analyses were performed in relatively few subjects. Fourth, the exact timing of cortisol sampling was not the same for all subjects and the early morning would have had our preference. Since this was not possible, we assessed fasting blood levels at term age, 3 and 6 months CA, as a second best option.[42] However, since there was no difference in mean sampling time between AGA and SGA subjects we considered this to have no influence on our results. In addition, despite our effort to standardize our salivary cortisol collection protocol by giving clear instructions and reporting time of sampling, it was not optimal, and the LLOQ limited the sensitivity of values in the lower ranges (i.e., ≤ 1 nmol/L). Considering the recently published guidelines of Stalder et al.,

the small number of samples to determine the CAR, as well as a single day of collection, may have influenced our results.[38]

CONCLUSION

In children born preterm, poor intrauterine and postnatal growth were associated with lower cortisol during early infancy, irrespective of gestational age. However, at age 8 years these differences were no longer present or could not be confirmed due to attrition. It is unknown whether alterations in HPA-axis activity in early infancy could attribute to increased health risks later in life.

REFERENCES

1. Watterberg KL. Adrenocortical function and dysfunction in the fetus and neonate. *Semin Neonatol*. 2004;9(1):13-21.
2. Finken MJ, van der Voorn B, Heijboer AC, de Waard M, van Goudoever JB, Rotteveel J. Glucocorticoid Programming in Very Preterm Birth. *Horm Res Paediatr*. 2016;85(4):221-231.
3. Ng PC. Effect of stress on the hypothalamic-pituitary-adrenal axis in the fetus and newborn. *J Pediatr*. 2011;158(2 Suppl):e41-43.
4. Bolt RJ, Van Weissenbruch MM, Popp-Snijders C, Sweep FG, Lafeber HN, Delemarre-van de Waal HA. Maturity of the adrenal cortex in very preterm infants is related to gestational age. *Pediatr Res*. 2002;52(3):405-410.
5. Kajantie E, Dunkel L, Turpeinen U, et al. Placental 11 beta-hydroxysteroid dehydrogenase-2 and fetal cortisol/cortisone shuttle in small preterm infants. *J Clin Endocrinol Metab*. 2003;88(1):493-500.
6. Duthie L, Reynolds RM. Changes in the maternal hypothalamic-pituitary-adrenal axis in pregnancy and postpartum: influences on maternal and fetal outcomes. *Neuroendocrinology*. 2013;98(2):106-115.
7. Cottrell EC, Seckl JR. Prenatal stress, glucocorticoids and the programming of adult disease. *Front Behav Neurosci*. 2009;3:19.
8. de Kloet ER, Claessens SE, Kentrop J. Context modulates outcome of perinatal glucocorticoid action in the brain. *Front Endocrinol (Lausanne)*. 2014;5:100.
9. Sebaai N, Lesage J, Vieau D, Alaoui A, Dupouy JP, Deloof S. Altered Control of the Hypothalamo-Pituitary-Adrenal Axis in Adult Male Rats Exposed Perinatally to Food Deprivation and/or Dehydration. *Neuroendocrinology*. 2002;76(4):243-253.
10. Guilloteau P, Zabielski R, Hammon HM, Metges CC. Adverse effects of nutritional programming during prenatal and early postnatal life, some aspects of regulation and potential prevention and treatments. *J Physiol Pharmacol*. 2009;60 Suppl 3:17-35.
11. Krishnaveni GV, Veena SR, Dhube A, Karat SC, Phillips DI, Fall CH. Size at birth, morning cortisol and cardiometabolic risk markers in healthy Indian children. *Clin Endocrinol (Oxf)*. 2014;80(1):73-79.
12. Phillips DI, Walker BR, Reynolds RM, et al. Low birth weight predicts elevated plasma cortisol concentrations in adults from 3 populations. *Hypertension*. 2000;35(6):1301-1306.
13. Embleton ND, Korada M, Wood CL, Pearce MS, Swamy R, Cheetham TD. Catch-up growth and metabolic outcomes in adolescents born preterm. *Arch Dis Child*. 2016;101(11):1026-1031.
14. Ong KK, Kennedy K, Castaneda-Gutierrez E, et al. Postnatal growth in preterm infants and later health outcomes: a systematic review. *Acta Paediatr*. 2015;104(10):974-986.
15. Amesz EM, Schaafsma A, Cranendonk A, Lafeber HN. Optimal growth and lower fat mass in preterm infants fed a protein-enriched postdischarge formula. *J Pediatr Gastroenterol Nutr*. 2010;50(2):200-207.
16. Gray JE, Richardson DK, McCormick MC, Workman-Daniels K, Goldmann DA. Neonatal therapeutic intervention scoring system: a therapy-based severity-of-illness index. *Pediatrics*. 1992;90(4):561-567.
17. Niklasson A, Ericson A, Fryer JG, Karlberg J, Lawrence C, Karlberg P. An update of the Swedish reference standards for weight, length and head circumference at birth for given gestational age (1977-1981). *Acta Paediatr Scand*. 1991;80(8-9):756-762.
18. Fredriks AM, van Buuren S, Burgmeijer RJ, et al. Continuing positive secular growth change in The Netherlands 1955-1997. *Pediatr Res*. 2000;47(3):316-323.
19. van de Lagemaat M, Rotteveel J, Lafeber HN, van Weissenbruch MM. Lean mass and fat mass accretion between term age and 6 months post-term in growth-restricted preterm infants. *Eur J Clin Nutr*. 2014;68(11):1261-1263.

20. Salazar A, Ojeda B, Duenas M, Fernandez F, Failde I. Simple generalized estimating equations (GEEs) and weighted generalized estimating equations (WGEEs) in longitudinal studies with dropouts: guidelines and implementation in R. *Stat Med*. 2016;35(19):3424-3448.
21. Fewtrell MS, Domellof M, Hojsak I, et al. Attrition in long-term nutrition research studies: a commentary by the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition early nutrition research working group. *J Pediatr Gastroenterol Nutr*. 2016;62(1):180-182.
22. Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology*. 2003;28(7):916-931.
23. McCalla CO, Nacharaju VL, Muneyyirci-Delale O, Glasgow S, Feldman JG. Placental 11 beta-hydroxysteroid dehydrogenase activity in normotensive and pre-eclamptic pregnancies. *Steroids*. 1998;63(10):511-515.
24. Aufdenblatten M, Baumann M, Raio L, et al. Prematurity is related to high placental cortisol in preeclampsia. *Pediatr Res*. 2009;65(2):198-202.
25. Osterholm EA, Hostinar CE, Gunnar MR. Alterations in stress responses of the hypothalamic-pituitary-adrenal axis in small for gestational age infants. *Psychoneuroendocrinology*. 2012; 37(10):1719-1725.
26. Schaffer L, Muller-Vizentini D, Burkhardt T, Rauh M, Ehlert U, Beinder E. Blunted stress response in small for gestational age neonates. *Pediatr Res*. 2009;65(2):231-235.
27. Clark PM, Hindmarsh PC, Shiell AW, Law CM, Honour JW, Barker DJ. Size at birth and adrenocortical function in childhood. *Clin Endocrinol (Oxf)*. 1996;45(6):721-726.
28. van Montfoort N, Finken MJ, le Cessie S, Dekker FW, Wit JM. Could cortisol explain the association between birth weight and cardiovascular disease in later life? A meta-analysis. *Eur J Endocrinol*. 2005;153(6):811-817.
29. Wust S, Entringer S, Federenko IS, Schlotz W, Hellhammer DH. Birth weight is associated with salivary cortisol responses to psychosocial stress in adult life. *Psychoneuroendocrinology*. 2005; 30(6):591-598.
30. Grunau RE, Tu MT, Whitfield MF, et al. Cortisol, behavior, and heart rate reactivity to immunization pain at 4 months corrected age in infants born very preterm. *Clin J Pain*. 2010; 26(8):698-704.
31. Grunau RE, Cepeda IL, Chau CM, et al. Neonatal pain-related stress and NFKBIA genotype are associated with altered cortisol levels in preterm boys at school age. *PLoS One*. 2013;8(9):e73926.
32. Brummelte S, Grunau RE, Zaidman-Zait A, Weinberg J, Nordstokke D, Cepeda IL. Cortisol levels in relation to maternal interaction and child internalizing behavior in preterm and full-term children at 18 months corrected age. *Dev Psychobiol*. 2011;53(2):184-195.
33. Buske-Kirschbaum A, Krieger S, Wilkes C, Rauh W, Weiss S, Hellhammer DH. Hypothalamic-pituitary-adrenal axis function and the cellular immune response in former preterm children. *J Clin Endocrinol Metab*. 2007;92(9):3429-3435.
34. Grunau RE, Haley DW, Whitfield MF, Weinberg J, Yu W, Thiessen P. Altered basal cortisol levels at 3, 6, 8 and 18 months in infants born at extremely low gestational age. *J Pediatr*. 2007;150(2):151-156.
35. Quesada AA, Tristao RM, Pratesi R, Wolf OT. Hyper-responsiveness to acute stress, emotional problems and poorer memory in former preterm children. *Stress*. 2014;17(5):389-399.
36. Brummelte S, Chau CM, Cepeda IL, et al. Cortisol levels in former preterm children at school age are predicted by neonatal procedural pain-related stress. *Psychoneuroendocrinology*. 2015; 51:151-163.
37. de Graaf J, van den Akker EL, van Lingen RA, et al. Five-year follow-up of effects of neonatal intensive care and morphine infusion during mechanical ventilation on diurnal cortisol rhythm. *J Pediatr*. 2014;165(3):459-463 e452.

38. Stalder T, Kirschbaum C, Kudielka BM, et al. Assessment of the cortisol awakening response: Expert consensus guidelines. *Psychoneuroendocrinology*. 2016;63:414-432.
39. Kajantie E, Hovi P. Is very preterm birth a risk factor for adult cardiometabolic disease? *Semin Fetal Neonatal Med*. 2014;19(2):112-117.
40. Hovi P, Vohr B, Ment LR, et al. Blood pressure in young adults born at very low birth weight: adults born preterm international collaboration. *Hypertension*. 2016;68(4):880-887.
41. Hofman PL, Regan F, Jackson WE, et al. Premature birth and later insulin resistance. *N Engl J Med*. 2004;351(21):2179-2186.
42. Spangler G. The emergence of adrenocortical circadian function in newborns and infants and its relationship to sleep feeding and maternal adrenocortical activity. *Early Hum Dev*. 1991;25: 197-208.