CLINICAL STUDY

Growth-restricted preterm newborns are predisposed to functional adrenal hyperandrogenism in adult life

Christiaan L Meuwese^{1,2}, Anne M Euser², Bart E Ballieux³, Huib A van Vliet⁴, Martijn J J Finken^{1,5}, Frans J Walther¹, Friedo W Dekker² and Jan M Wit¹

¹Department of Pediatrics, ²Department of Clinical Epidemiology, ³Chemical Laboratory and ⁴Department of Reproductive Medicine, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, The Netherlands and ⁵Department of Pediatrics, VU University Medical Center, PO Box 7057, MB Amsterdam, The Netherlands

(Correspondence should be addressed to F W Dekker; Email: f.w.dekker@lumc.nl)

Abstract

Background: The long-term effects of perinatal growth and corticosteroid exposure on adrenal steroid concentrations in adults born very preterm are uncertain.

Objectives: To examine the effect of birth weight, early postnatal growth, and pre- and postnatal corticosteroid administration on serum adrenal steroids in 19-year-old subjects born very preterm.

Design and methods: Subjects born before 32 weeks of gestation in The Netherlands participating in the Project on Preterm and Small for Gestational Age Infants (POPS) were investigated at 19 years of age. Serum cortisol, DHEA sulfate (DHEAS), and androstenedione (Adione) concentrations were measured in 393 out of 676 eligible subjects, compared with controls, and associated with perinatal growth and pre- and postnatal corticosteroids administration using multiple linear regression analyses.

Results: Serum DHEAS and Adione in men and women were higher than in controls. In the multiple regression analyses, birth weight SDS showed a statistically significant negative association with serum DHEAS concentrations in women (β : -0.865 , 95% confidence interval (CI): -1.254 to -0.476) and in men (β : -0.758 , 95% CI: -1.247 to -0.268) and with serum Adione concentrations in women (β : -0.337 , 95% CI: -0.593 to -0.082). Early postnatal weight gain showed no association with any of measured adrenal markers. In women, serum Adione was associated with postnatal dexamethasone exposure (β : 0.932 , 95% CI: 0.022 – 1.843).

Conclusions: Young adults born very preterm show elevated adrenal androgens, particularly when born small for gestational age. Postnatal corticosteroid administration is positively associated with serum Adione in young women.

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Introduction

According to the hypothesis now called ‘Developmental origins of health and disease’, intrauterine growth retardation, and as a consequence low birth weight for gestational age as well as fast postnatal catch-up growth predisposes to raised blood pressure, cardiovascular disease, type 2 diabetes mellitus, and insulin resistance in adulthood (1, 2). A recent meta-analysis suggests that elevated cortisol concentrations may explain part of this association, at least in individuals born at term (3).

Moreover, for the adrenal steroids DHEA sulfate (DHEAS) and androstenedione (Adione), there are indications that intrauterine growth retardation is associated with long-term changes in adult serum concentrations. Various studies have shown that low birth weight is associated with higher serum DHEAS concentrations, and as a consequence predisposes to an earlier adrenarche and/or an adrenal hyperandrogenic

profile in adult life (4–7), though in other studies, no such association was observed (8, 9). An early adrenarche appears to be associated with a higher risk of hyperandrogenism and polycystic ovaries (10, 11). Furthermore, associations have been found between early postnatal growth and adrenal function in later life (12). In the only study including young adults born preterm (<32 weeks), serum cortisol concentrations in both genders and DHEA, DHEAS, and Adione in women were elevated as compared with control subjects, the latter three being positively correlated with insulin concentrations (7).

Mothers with premature contractions are frequently treated, if time permits, with corticosteroids to stimulate fetal lung maturation (13), and occasionally, very preterm infants need corticosteroids to wean them from mechanical ventilation (14). It is unclear what the long-term consequence are of these therapeutic interventions. In experimental animal studies, corticosteroid administration during the last week of pregnancy

induces permanent alterations in glucocorticoid activity in the rat and eventually leads to hypertension (15). In young human infants, a brief period of steroid exposure in the first weeks after birth resulted in a mild transient adrenal suppression (16, 17). In another study, cortisol concentrations were higher in individuals exposed to betamethasone prenatally compared with nonexposed subjects at 30 years of age. However, this difference disappeared after adjustment for confounding factors (18). The long-term effects of postnatal corticosteroid administration are unknown.

Since the long-term effects of preterm extrauterine growth retardation appear to be similar to those of intrauterine growth retardation (19), we hypothesized that prenatal and early postnatal growth and perinatal corticosteroid administration may affect serum adrenal steroid concentrations in early adulthood in individuals born very preterm. We tested this in 19-year-old participants of the Project on Preterm and Small for Gestational Age Infants (POPS).

Subjects and methods

Subjects

The POPS is a nationwide multicenter study on a birth cohort, comprising 94% of the very preterm (<32 weeks of gestation) and/or very low birth weight infants (<1500 g) born alive in the Netherlands in 1983. In 2002, all eligible subjects aged 19 years were approached by mail to participate in the POPS-19 study for which the approval of the medical ethics committees of all participating centers was obtained. For this study, only those subjects with a gestational age <32 weeks were studied. Subjects with congenital malformations leading to changes in body proportions and body composition were not eligible for inclusion, nor subjects who used medication that could influence serum steroid concentrations.

Subjects who gave written informed consent to participate and met the inclusion criteria were seen after an overnight fast between 0830 and 1000 h at one of the outpatient clinics of the ten participating centers. After a medical history and physical examination, a venous blood sample was obtained after 30 min in a supine position. Assessors were blinded with respect to perinatal characteristics and perinatal corticosteroid exposure of all subjects.

The findings on growth (20) and cognitive status (21), as well as differences between participating and nonparticipating subjects (22) have been reported previously. Gestational age was assessed by the mother's last day of menstruation. Details about birth characteristics, growth, and other characteristics have been collected from birth onward, and growth and insulin resistance at 19 years were reported previously (2, 20). Birth size was expressed as SDS for gestational age

and gender using Swedish references (23), and postnatal growth parameters were expressed as SDS for age and gender using Dutch references (24, 25). Early postnatal weight gain (EPWG) was defined as the difference between weight SDS at 3 months (corrected for gestational age) and weight SDS at birth (Δ SDS). This parameter was the result of an initial period of growth retardation in most cases, followed by a variable degree of catch-up growth. Apgar scores, determined 5 min after birth, were categorized as high (≥ 6) or low (< 6).

In 1983, there was no firm scientific basis for administering antenatal betamethasone, and its use was not generally adopted. In practice, some obstetricians refrained from prescribing glucocorticoids prenatally, while others were used to prescribe them on a more regular basis (26). Thus, some pregnant women with impending preterm delivery were treated with two doses of 12 mg betamethasone, given 12 h apart, prior to birth to accelerate fetal lung maturation, while others did not receive corticosteroids. Inferentially, the allocation of prenatal betamethasone to participants of the POPS study was neither based on maternal nor based on fetal characteristics, but only on different general attitudes toward this form of treatment in the various health centers. The same situation applied to the use of corticosteroids administered postnatally.

To obtain reference values, serum DHEAS and Adione were determined with the same assay in serum samples from 80 healthy volunteers (40 women and 40 men) aged between 18 and 25 years participating as healthy controls in two studies from the Genealogical Department (courtesy of Prof. F Helmerhorst) and the Centre for Human Drug Research (courtesy of Dr J Burggraaf). As cortisol values in the morning are largely dependent on variables as stress, time of awakening, and use of oral contraceptives (OAC), comparison with general reference values is of limited value and is not studied in this group.

Analytical methods

All blood samples were stored at -80°C and thawed only once immediately before analysis. Cortisol was measured with a fluorescence polarization immunoassay on an Abbott TDx (Abbott Laboratories). The sensitivity of this assay is 20 nmol/l, and the interassay coefficient of variation (CV) ranged from 3.1 to 6.4% at different concentrations.

DHEAS was measured using a luminescence immunoassay on an Immulite 2500 analyser (Siemens Diagnostics, Breda, the Netherlands). Variation coefficients ranged from 4.3 to 9.6% over a concentration range of 1.1–7.8 $\mu\text{mol/l}$. Adione was measured using a RIA of Diagnostic Systems Laboratories (DSL 4200, Beckman Company, Sinsheim, Germany). CV approximated 10% over the clinical range of 3–20 nmol/l.

Insulin (mU/l) and glucose (mmol/l) were measured, and homeostasis model assessment-insulin resistance (HOMA-IR) was calculated as $(\text{glucose} \times \text{insulin}) / 22.5$ (2).

Statistical analysis

Statistical analyses were performed in SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Sex differences were calculated using a Student's *t*-test and a Mann–Whitney *U* test for normally and nonnormally distributed variables respectively and a χ^2 -test for the dichotomous variables. Multivariate linear regression analyses were used to obtain regression coefficients. For all tests, a 95% confidence interval not including zero was considered statistically significant. Multiple pregnancy and socioeconomic status (SES) were included as confounders since they were both associated with perinatal characteristics and concentrations of adrenal hormone concentrations in later life (27, 28). SES was classified on a six-point scale in which > 2 was labeled high and ≤ 2 was labeled low. In addition, center of care was converted to a dummy variable and added to the model as a covariate to adjust for potential confounding

caused by center-specific characteristics. Other variables were not included as confounders because of their potential role in the causal pathway between birth weight and serum hormone concentrations at 19 years of age. In the crude analysis of the multiple regression analysis, EPWG was already adjusted for birth weight. In addition, in women, we tested for interaction between phase of the menstrual cycle and use of OAC and the primary determinants (birth weight SDS, EPWG, prenatal betamethasone exposure, and postnatal betamethasone exposure) on hormone concentrations at age 19. As no interaction was present, the use of OAC was added as a binary covariate in all regression analyses for female participants.

In additional regression analyses, we analyzed the effect of serum insulin and C-peptide concentrations and HOMA-IR on serum cortisol, Adione, and DHEAS concentrations. Since the distribution of HOMA-IR, C-peptide, and insulin was not normally distributed, the logarithmic transformed values were included as products in the regression analyses. To investigate to what extent this effect was confounded by the effect of perinatal characteristics, we corrected for all

Table 1 Perinatal characteristics and findings at 19 years of age in POPS-19 subjects.

	Men (<i>n</i> =189)	Women (<i>n</i> =204)	<i>P</i> value
Perinatal characteristics ^a			
Gestational age (weeks) ^b	29.7 (1.5)	29.8 (1.5)	0.714
Birth weight (g) ^b	1383 (331)	1273 (328)	0.001
Birth weight (SDS) ^b	−0.03 (0.94)	−0.19 (1.07)	0.114
Weight at 3 m post term (g) ^b	5335 (940)	4949 (813)	0.0001
Weight at 3 m post term (SDS) ^b	−1.05 (1.69)	−0.93 (1.25)	0.427
EPWG (Δ SDS) ^{b,c}	−1.00 (1.60)	−0.71 (1.07)	0.046
IRDS history ^{d,e}	99 (52.4)	93 (45.6)	0.178
Multiple birth ^e	47 (24.9)	47 (23.0)	0.671
Prenatal betamethasone ^e	48 (25.4)	31 (15.2)	0.012
Postnatal dexamethasone ^e	16 (8.5)	18 (8.8)	0.900
Low Apgar score ^{e,f}	27 (14.3)	18 (8.8)	0.117
Caucasian ^e	162 (85.7)	181 (88.7)	0.371
Low SES ^{e,g}	66 (34.9)	69 (34.3)	0.902
Characteristics at 19 years ^h			
Weight SDS ^b	−0.43 (1.24)	−0.52 (1.41)	0.483
Height SDS ^b	−0.59 (1.12)	−0.59 (1.10)	0.984
BMI (kg/m ²) ^{b,i}	21.7 (3.1)	21.7 (3.4)	0.992
Waist circumference (SDS) ^b	0.70 (0.98)	0.24 (1.19)	<0.0001
Oral contraceptives ^e		136 (67.0)	–
Follicular phase ^{e,j}		35 (53.8)	–
Glucose (mmol/l) ^b	5.19 (0.42)	4.81 (0.37)	<0.0001
Insulin (mU/l) ^k	8.0 (6.0–11.0)	9.0 (7.0–11.0)	0.490
C-peptide (mmol/l) ^k	0.62 (0.50–0.80)	0.66 (0.55–0.81)	0.068
HOMA-IR ^k	1.92 (1.42–2.70)	1.84 (1.39–2.49)	0.273

^aSome characteristics were not measured in a maximum of 28 participants.

^bMean (s.d.) differences were tested by means of an unpaired *t*-test.

^cEPWG, early postnatal weight gain was defined by weight SDS at 3 months post term minus weight SDS at birth.

^dIRDS, infant respiratory distress syndrome.

^eCategorical variable, *n* (%), differences were tested by means of a χ^2 -test.

^fLow Apgar score after 5 min was defined by a score < 6 .

^gSES, socioeconomic status was classified on a six-point scale in which > 2 was high and ≤ 2 was low.

^hSome characteristics were not measured in a maximum of eight participants.

ⁱBMI, body mass index was calculated as weight divided by squared height (m²).

^jPart of women not using oral contraceptives who were in the follicular phase of the menstrual cycle.

^kMedian (IQR) differences were tested by means of a Mann–Whitney *U* test.

aforementioned perinatal variables in a multivariate variant of these models.

Separate regression analyses were performed to test for interaction between birth weight SDS and EPWG and for interaction between corticosteroid exposure prenatally and postnatally. In these two models, we included the product of birth weight SDS and EPWG and the product of prenatal betamethasone and postnatal dexamethasone administration respectively as an interaction term.

Results

In 1983, 1012 individuals in the POPS cohort were born at <32 weeks of gestation. Before the age of 19 years, 336 subjects died. Consequently, 676 individuals were eligible for this study, of which 178 individuals were lost to follow-up (response rate 73.7%). In 105 participants, no laboratory analyses could be performed, resulting in a total of 393 individuals who were included in the statistical analyses in this study. Comparing the study group with the subjects who did not participate in the follow-up study ($n=178$) revealed that male sex, lower SES, non-Caucasian origin, and a higher morbidity were independently associated with nonresponse. Birth weight SDS, gestational age, and perinatal corticosteroid administration were not significantly different between the response group and nonresponse group (data not shown). The 105 subjects from whom no blood could be withdrawn were not significantly different with respect to the baseline parameters from those included in the current analysis. None of the subjects reported hirsutism, and none used medication that could affect serum prolactin concentrations. Two subjects used L-thyroxine treatment for hypothyroidism.

For the reference group in which Adione and DHEAS concentrations were determined, mean (s.d.) age was 19.5 (1.0), 21.8 (1.4), and 21.8 (2.0) years for women using OAC, women not using OAC, and men respectively.

Table 1 shows characteristics of the subjects in the perinatal phase and at follow-up. Mean gestational age was ~30 weeks. Mean birth weight was close to 1300 g, slightly higher in men than in women, but when expressed as SDS, mean birth weight was equal in both genders (close to -0.1 SDS). Weight at 3 months post term was ~ -1 SDS, with EPWG being more compromised in men than in women. Prenatal betamethasone administration was more frequent in men than in women. At 19 years of age, height and weight SDS were similar in women and men, and lower compared to population references. Waist circumference was higher in men, and was higher in both genders than in the general population, as earlier reported (19). Mean fasting glucose concentrations were higher in males than in females, but median insulin, C-peptide, and HOMA-IR were similar in both genders.

In Table 2, serum cortisol, DHEAS, and Adione are shown for men and women (with or without OAC) in POPS subjects and healthy controls. The use of OAC is associated with a considerable increase in cortisol concentrations, and a decrease in DHEAS and Adione. In women not using OAC, serum hormone concentrations did not differ significantly between individuals in follicular or luteal phase of the menstrual cycle (data not shown). After excluding women using OAC, serum cortisol was not different between genders. Serum DHEAS concentrations were lower in women without OAC than in men in POPS subjects, and even lower in women using OAC. In all three groups, DHEAS was higher in POPS subjects compared to controls. Adione was highest in women not using OAC, followed by men and women using OAC. Adione in POPS men was significantly higher than controls, and in POPS women without OAC, it was significantly lower.

In Tables 3–5, results of the multiple regression analyses for serum cortisol, DHEAS, and Adione versus early growth and perinatal corticosteroid exposure are presented. For cortisol (Table 3), no association was observed between serum concentrations and birth weight, EPWG, and perinatal corticosteroid administration. For DHEAS (Table 4), in both men and women,

Table 2 Serum hormone levels within subgroups of POPS subjects at 19 years of age.

	Men		Women			
	POPS ($n=189$) ^a	Reference ($n=40$)	Oral contraceptives		No oral contraceptives	
			POPS ($n=136$) ^a	Reference ($n=20$)	POPS ($n=67$) ^a	Reference ($n=20$)
Cortisol ($\mu\text{mol/l}$) ^b	0.40 (0.11)		0.90 (0.29)		0.37 (0.14)	
DHEAS ($\mu\text{mol/l}$) ^b	9.33 (3.21)*	7.65 (2.95)	5.80 (2.56)*	4.44 (2.18)	8.42 (3.61)*	6.37 (2.37)
Androstenedione (nmol/l) ^b	5.31 (1.56)*	4.69 (1.54)	4.60 (1.77)	5.22 (2.19)	6.21 (2.08)*	7.27 (1.98)

*Statistically significant difference between POPS subjects and reference subjects ($P<0.05$).

^aNot available in a maximum of four subjects.

^bSerum hormone concentrations are presented as mean (s.d.), and differences between POPS individuals and reference subjects were tested by an unpaired t -test.

Table 3 Multiple regression analysis for serum cortisol levels.

	Women (<i>n</i> =204) ^a		Men (<i>n</i> =189) ^a	
	Crude β (95% CI) ^b	Adjusted β (95% CI) ^c	Crude β (95% CI)	Adjusted β (95% CI) ^c
Birth weight (SDS)	−0.003 (−0.037 to 0.031)	−0.005 (−0.041 to 0.031)	0.000 (−0.018 to 0.017)	−0.000 (−0.019 to 0.018)
EPWG (Δ SDS) ^d	0.023 (−0.012 to 0.058)	0.035 (−0.002 to 0.072)	−0.002 (−0.012 to 0.009)	−0.000 (−0.012 to 0.010)
Prenatal beta ^e	−0.001 (−0.098 to 0.099)	0.001 (−0.098 to 0.099)	−0.024 (−0.061 to 0.013)	−0.042 (−0.084 to 0.000)
Postnatal dexam ^f	−0.004 (−0.127 to 0.118)	−0.010 (−0.135 to 0.114)	0.055 (−0.003 to 0.113)	0.054 (−0.008 to 0.117)

^aSome characteristics were measured in a slightly fewer participants.^bThe crude analysis for women was also adjusted for the use of oral contraceptives.^cAdjusted for gestational age, SES, multiple pregnancy, center of care, and use of oral contraceptives in women.^dEPWG, early postnatal weight gain was defined by weight SDS at 3 months minus birth weight SDS. In the crude analysis, EPWG was already corrected for birth weight SDS.^ePrenatal beta, prenatal betamethasone administration was not corrected for birth weight SDS in the adjusted analysis.^fPostnatal dexam, postnatal dexamethasone administration was also corrected for prenatal betamethasone administration and birth weight SDS.

a statistically significant inverse association was found with birth weight, persisting after adjustment for confounders. EPWG, prenatal betamethasone administration, and postnatal dexamethasone administration showed no association with serum DHEAS concentrations at 19 years of age in both men and women. Concerning serum Adione concentrations (Table 5), birth weight showed a statistically significant negative association in women and a negative trend in men. In women, prenatal betamethasone administration after adjustment and postnatal dexamethasone administration were positively correlated with serum Adione concentrations. In men, no trend was observed between perinatal corticosteroid exposure and serum Adione concentrations at 19 years of age. Testing for interaction between birth weight SDS and EPWG and for interaction between corticosteroid exposure prenatally and postnatally in association with adrenal hormone concentrations did not alter our findings substantially (data not shown). Moreover, adjustment for waist circumference did not significantly change the results found (data not shown).

In Table 6, the associations between serum insulin and C-peptide concentrations and HOMA-IR versus serum DHEAS are shown. In women not using OAC, a positive trend was observed with DHEAS, but after adjustment for perinatal characteristics, betas became

close to zero. Concerning cortisol and Adione, point estimates were centered around zero and were nonsignificant for both male and female POPS subjects (data not shown).

Discussion

In this prospective study in young adults born very preterm, serum DHEAS concentrations were increased as compared with controls and negatively associated with birth weight in both genders. Serum Adione concentrations in men were increased but not associated with perinatal corticosteroid exposure, while in women, they were decreased and positively associated with perinatal corticosteroid exposure. In both genders, Adione was negatively associated with birth weight SDS. Serum cortisol did not show any association with early growth nor corticosteroid exposure. EPWG (weight SDS 3 months post term minus birth weight SDS) was not associated with any of the measured adrenal hormones. In women not using OAC, the association between insulin, C-peptide, and HOMA-IR versus DHEAS showed a positive trend, which disappeared after adjustment for perinatal characteristics.

The increased DHEAS concentrations in very preterm born young adults, in combination with the negative

Table 4 Multiple regression analysis for serum DHEA sulfate levels.

	Women (<i>n</i> =200) ^a		Men (<i>n</i> =185) ^a	
	Crude β (95% CI) ^b	Adjusted β (95% CI) ^c	Crude β (95% CI)	Adjusted β (95% CI) ^c
Birth weight (SDS)	−0.865 (−1.254 to −0.476)	−0.889 (−1.298 to −0.481)	−0.758 (−1.247 to −0.268)	−0.799 (−1.320 to −0.277)
EPWG (Δ SDS) ^d	0.289 (−0.114 to 0.692)	0.378 (−0.052 to 0.808)	0.058 (−0.249 to 0.364)	−0.005 (−0.346 to 0.336)
Prenatal beta ^e	0.699 (−0.471 to 1.870)	1.000 (−0.191 to 2.191)	−0.212 (−1.283 to 0.859)	−0.050 (−1.148 to 1.249)
Postnatal dexam ^f	−0.418 (−1.860 to 1.024)	−0.375 (−1.798 to 1.047)	−0.799 (−2.504 to 0.906)	−0.142 (−1.925 to 1.642)

^aSome characteristics were measured in a slightly fewer participants.^bThe crude analysis for women was also adjusted for the use of oral contraceptives.^cAdjusted for gestational age, SES, multiple pregnancy, center of care, and use of oral contraceptives in women.^dEPWG, early postnatal weight gain was defined by weight SDS at 3 months minus birth weight SDS. In the crude analysis, EPWG was already corrected for birth weight SDS.^ePrenatal beta, prenatal betamethasone administration was not corrected for birth weight SDS in the adjusted analysis.^fPostnatal dexam, postnatal dexamethasone administration was also corrected for prenatal betamethasone administration and birth weight SDS.

Table 5 Multiple regression analysis for serum androstenedione levels.

	Women (n=200) ^a		Men (n=185) ^a	
	Crude β (95% CI) ^b	Adjusted β (95% CI) ^c	Crude β (95% CI)	Adjusted β (95% CI) ^c
Birth weight (SDS)	-0.337 (-0.593 to -0.082)	-0.266 (-0.531 to -0.002)	-0.200 (-0.442 to 0.042)	-0.171 (-0.430 to 0.088)
EPWG (Δ SDS) ^d	0.144 (-0.121 to 0.408)	0.129 (-0.153 to 0.412)	0.017 (-0.131 to 0.165)	0.067 (-0.100 to 0.234)
Prenatal beta ^e	0.580 (-0.165 to 1.324)	0.856 (0.118 to 1.594)	-0.376 (-0.894 to 0.142)	-0.408 (-0.988 to 0.173)
Postnatal dexta ^f	0.932 (0.022 to 1.843)	1.318 (0.421 to 2.214)	0.177 (-0.653 to 1.008)	0.381 (-0.500 to 1.262)

^aSome characteristics were measured in a slightly fewer participants.

^bThe crude analysis for women was also adjusted for the use of oral contraceptives.

^cAdjusted for gestational age, SES, multiple pregnancy, center of care, and use of oral contraceptives in women.

^dEPWG, early postnatal weight gain was defined by weight SDS at 3 months minus birth weight SDS. In the crude analysis, EPWG was already corrected for birth weight SDS.

^ePrenatal beta, prenatal betamethasone administration was not corrected for birth weight SDS in the adjusted analysis.

^fPostnatal dexta, postnatal dexamethasone administration was also corrected for prenatal betamethasone administration and birth weight SDS.

association with birth weight SDS in the POPS subjects, suggest that intrauterine growth of very preterm born individuals affects serum DHEAS concentrations. Our results concerning DHEAS are similar to those of the only previous study in individuals born under 32 weeks of gestation (7). However, since in that study birth weight was not corrected for gestational age, it is likely that the association between birth weight and serum concentrations is, at least partly, confounded by gestational age. The results of our study and the previous study in very preterm born subjects are supported by several studies in individuals born > 32 weeks of gestation (4–6, 12, 29–31), suggesting that programming of the hypothalamic–pituitary–adrenal (HPA) axis starts before 32 weeks. This is consistent with the observation that the adrenals are already able to respond to exogenous stimulation with CRH or ACTH at 26 weeks of gestation (16, 17, 32). The role of growth retardation on adrenal programming between 32 and 40 weeks could not be investigated in our study, because no weight SDS at term was available.

The crude positive trend between fasting log insulin concentrations, log C-peptide concentrations, and HOMA-IR versus serum DHEAS in women, particularly women not using OAC, is in line with results obtained in the previous study conducted in preterm born individuals (7). However, after adjustment for perinatal characteristics, betas were markedly reduced. We speculate that both insulin resistance and adrenal hyperandrogenism are caused by aberrant perinatal

growth in preterms, or that preterm birth causes insulin resistance, which in turn causes overproduction of DHEAS. The latter hypothesis is supported by observations that insulin overall stimulates P450c17a activity through increased 17-hydroxylase activity, which is only partially compensated by a decreased 17,20-lyase activity (33). A third possibility is that corticosteroid-binding globulin (CBG) may play a role in the elevated DHEAS concentrations (34).

The situation with respect to Adione seems to be more complex, because Adione in women is produced for ~50% in the adrenals, and the remainder in the ovaries (35). In men, the majority of Adione is produced in the adrenals, and a smaller part in the testes (35, 36). Our finding that serum Adione in female participants in POPS is lower than in female controls, in contrast to the elevated DHEAS concentrations, would suggest that gonadal production of Adione may be decreased, or that the conversion of DHEA to Adione is decreased in the adrenal. Interestingly, there was a significant negative association with birth weight SDS, suggesting that the adrenal component may still increase with decreasing birth weight. In men, Adione was elevated in preterm born individuals, and negatively associated with birth weight SDS, although not statistically significant. In the previous study in preterm born individuals (7), Adione was elevated in both genders, but negatively correlated with birth weight only in women. In that study, and consistent with our findings, no association was found with serum insulin

Table 6 Multiple regression analysis for measures of insulin resistance and serum DHEA sulfate (DHEAS) levels, stratified for the use of oral contraceptives (OAC).

	Women without oral contraceptives (n=65) ^a		Women with oral contraceptives (n=134) ^a	
	Crude β (95% CI)	Adjusted β (95% CI) ^b	Crude β (95% CI)	Adjusted β (95% CI) ^b
Log (HOMA-IR)	2.580 (-1.876 to 7.036)	0.158 (-4.837 to 5.153)	0.387 (-2.134 to 2.908)	0.075 (-2.334 to 2.485)
Log (insulin)	3.092 (-1.422 to 7.605)	0.812 (-4.379 to 6.004)	-0.303 (-3.015 to 2.408)	-0.359 (-2.901 to 2.183)
Log (C-peptide)	4.223 (-2.085 to 10.531)	1.211 (-5.517 to 7.938)	0.559 (-2.602 to 3.719)	0.645 (-2.430 to 3.721)

^aOut of the total of 203 women, in 2 subjects not using OAC and in 2 subjects using OAC, serum DHEAS was not measured.

^bAssociations were adjusted for birth weight, gestational age, SES, multiple pregnancy, center of care, use of oral contraceptives in women, prenatal betamethasone administration, and postnatal dexamethasone administration.

concentrations, although insulin and insulin-like growth factors appear to stimulate the LH-induced androgen production in ovarian theca cells (37, 38).

For all steroids, there were differences between men, women without OAC, and women using OAC. Cortisol in women using OAC was much higher than the other groups, which could be attributed to an elevated concentration of CBG induced by estrogens (39, 40). The lower DHEAS concentrations in women than in men, and still lower concentrations in women using OAC, can be explained by the increased metabolic clearance induced by estrogens (41, 42). This observation is supported by a randomized controlled trial in which introduction of OAC lowered adrenal androgen concentrations substantially (43). The lower Adione concentrations in men as compared with concentrations in women not using OAC can be explained by the lower Adione production in the testes than in the ovaries. The Adione suppression by OAC is due to the suppression of ovarian steroid production by the inhibition of gonadotropins, particularly LH (44).

In association with perinatal corticosteroid exposure in women, only serum Adione concentrations displayed a statistically significant positive association with postnatal dexamethasone administration and (after adjustment) also with prenatal betamethasone administration. The apparent sex difference in the sensitivity to steroid exposure is in line with observations in the rat: the response of the HPA axis in the female rat to stress is much more profound than the response observed in males (45).

A possible limitation of this study is that the substantial number of loss to follow-up (22), which could have introduced bias by nonresponse. However, as birth weight, weight at 3 months, and perinatal corticosteroid administration were not different between responders and nonresponders, confounding is unlikely. Furthermore, because perinatal corticosteroid administration was not randomly assigned, selection bias could have been introduced. However, Vandenbroucke argued that in case of unknown consequences of a therapy modality, allocation of that therapy is independent of these consequences and therefore random (46). Concerning perinatal corticosteroid administration in the 1980s, long-term consequences, and especially the effect on serum adrenal hormone concentrations, were not known (26). Therefore, allocation of corticosteroids in POPS may be considered to be random. Finally, since phenotypic parameters (such as a semiobjective assessment of hirsutism, e.g. a Ferriman–Gallwey score) of adrenal hyperactivity were not recorded in this study, the clinical relevance of the biochemical findings remains uncertain.

In conclusion, adults born very preterm, in particular women with a low birth weight for gestational age or who received corticosteroids perinatally, may be at increased risk of the consequences of adrenal hyperandrogenism.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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