Adrenal androgen trajectories are established during childhood in preterm boys

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Abbreviations: DHEAS, dehydroepiandrosterone sulphate; IQR, interquartile range; SDS, standard deviation score; SGA, small for gestational age; WHR, waist-to-height ratio.

Abstract
Aim: We investigated longitudinal adrenal androgen concentrations and any relationship between gestational age, birth size, anthropometric parameters and adrenal androgen concentrations during childhood in boys born moderate to late preterm.

Methods: This longitudinal, prospective study included 58 boys born at 32+0 to 36+6 weeks of gestation. Dehydroepiandrosterone sulphate and androstenedione were analysed by liquid chromatography-tandem mass spectrometry, and anthropometric data were recorded from 5 to 10 years of age.

Results: Dehydroepiandrosterone sulphate concentrations correlated with weight standard deviations scores (SDS) from 7 to 10 years of age and waist-to-height ratios at seven and 10 years of age. Androstenedione correlated with weight SDS from 7 to 10 years of age and waist-to-height ratios at 10 years of age. Longitudinal analysis showed a relationship between weight SDS and waist-to-height SDS and dehydroepiandrosterone sulphate (p < 0.001 and p < 0.001, respectively) and androstenedione (p = 0.002 and p = 0.003, respectively), independently of age.

Conclusion: The trajectories of anthropometric parameters and adrenal androgen secretion were consistent from 5 to 10 years of age in this cohort. The body composition reflected by current weight and the waist-to-height ratio, rather than gestational age and birth size, was associated with adrenal androgen secretion.
1 | INTRODUCTION

Dehydroepiandrosterone sulphate (DHEAS) is the best serum marker of adrenal androgen secretion.\(^1\) Androstenedione also originates from the adrenal gland.\(^2\) Antenatal stress is believed to increase the risk of both preterm birth and low birth weight. It has also been shown to affect adrenal androgen secretion in later life, by influencing the hypothalamus-pituitary-adrenal axis through early life programming.\(^3\)\(^-\)\(^5\) Low birth weight has been associated with premature adrenarche and increased risks of developing metabolic syndrome in adult life.\(^1\) However, studies have suggested that the postnatal catch-up growth during infancy and childhood is even more potent in increasing adrenal activity compared with birth weight in children born small for gestational age (SGA).\(^6\)\(^7\) A relationship between lower birth weight and more pronounced childhood adrenal androgen secretion is also found in children with normal birth weights.\(^8\) Adrenal androgen secretion in healthy children has also been related to both current weight and catch-up in weight standard deviation score (SDS) from birth to three years of age.\(^8\) Furthermore, increased sex steroid hormone secretion has been found to induce increased insulin resistance, which promotes growth, and changed body composition.\(^7\) There has been a lack of longitudinal studies of adrenal androgen secretion during childhood, but large cross-sectional studies have reported that both DHEAS and androstenedione increase with age.\(^9\)\(^10\)

Our research formed part of an ongoing, prospective, single-centre study that investigated the relationship between gestational age, birth size, anthropometric parameters and adrenal androgen secretion during childhood. In this paper, we evaluate the longitudinal adrenal androgen secretion patterns of DHEAS and androstenedione, as well as growth patterns of 58 boys who were born moderate to late preterm. The investigations were carried out when they reached 5–10 years of age. We aimed to investigate adrenal androgen concentrations and any relationship between adrenal androgens and gestational age, as well as anthropometric data at birth and during childhood.

2 | MATERIALS AND METHODS

2.1 | Study cohort

The study population comprised 58 boys born moderate to late preterm at 32+0 to 36+6 weeks of gestation from September 2002 to June 2004 at Sahlgrenska University Hospital, Gothenburg, Sweden. It was part of a larger cohort of 247 children (137 boys) from a prospective, longitudinal, population-based study. Children with syndromes, chromosomal abnormalities, severe malformations or other chronic diseases were excluded at the start of the study.\(^11\) The parents of the 66 boys who were still part of the study at five years of age were invited to take part in this follow-up study, and parents of 58 boys agreed to further participation.\(^12\) We collected blood samples on at least two occasions for every subject except for one boy who only provided a sample at the age of five. All 58 boys had complete birth data and anthropometric data at 5 years of age, but at 10 years of age, 10 were lost to follow-up. The mothers of 12 boys (21%) had preeclampsia, and one had hypertonia. There were eight (14%) boys born SGA, defined as birth weight below –2 SDS,\(^13\) including three boys born to mothers with preeclampsia.

2.2 | Study design

Birth weight SDS and birth length SDS were calculated based on previously reported data\(^11\) and the Swedish reference for newborn infants.\(^13\) Anthropometric data were recorded yearly from 5-8 and at 10 years of age using three separate measurements. Mean values were calculated and transformed to an SDS.\(^14\) Body weight was measured using a SECA 701 electronic step scale (Seca Deutschland) with the child just wearing underwear. Standing height was measured using a wall-mounted Ulmer stadiometer (Busse Design and Engineering GmbH). Waist circumference was measured in centimetres at the umbilical level, in a standing position, using an inelastic measuring tape. The waist-to-height ratio (WHtR) was calculated by dividing the waist circumference by height in centimetres. Pubic hair was recorded at eight and 10 years of age, according to the Tanner stages.\(^15\)

The boys underwent an overnight fasting oral glucose tolerance test at 5 years of age, by drinking a solution of 0.3 g glucose/ml, corresponding to 1.75 g/kg. Blood glucose was registered at the start of the test and after 30, 60 and 120 min. Impaired glucose tolerance was defined as a two-hour post-load blood glucose of >7.8 mmol/L.\(^16\)

Venous blood samples were taken to analyze DHEAS and androstenedione from the age of five years. These were collected at 8–11 am, in each study year. The serum was separated from the samples and stored at −80°C until they were assayed. In a total of six cases, blood sampling could not be carried out at the first attempt, but three were successfully repeated within six months and new anthropometric parameters were collected from these boys for comparison purposes.
Serum DHEAS and androstenedione concentrations were simultaneously determined by liquid chromatography-tandem mass spectrometry using the Agilent 6460 (Agilent Technologies). The lower limit of detection was 0.1 $\mu$mol/L for DHEAS and 0.1 nmol/L for androstenedione. The total coefficient of variation was 8% at 0.4 $\mu$mol/L and 4% at 1.1 $\mu$mol/L for DHEAS and 21% at 0.2 nmol/L and 12% at 1.2 nmol/L for androstenedione.

Blood glucose was analysed using the HemoCue 201 RT (HemoCue AB, Ängelholm, Sweden). The inter-assay coefficient of variation was 2.1% at 6 mmol/L.

Descriptive data are expressed as the medians, interquartile ranges and ranges. Hormone concentrations below the limit of detection were set to half the limit. Pearson’s correlation coefficient (r) was calculated to assess univariate correlations between adrenal androgens at 5–8 and 10 years of age and birth data and anthropometric data. For each set of correlations at five different ages, the significance was interpreted in a multiple inference context using Holm-Bonferroni. A $p$-value < 0.05 was considered statistically significant.

SPSS version 26 (IBM Corp) and R version 3.6.1 were used for the statistical analyses.

2.5 | Ethics

The study population was born at a median of 35 + 5 weeks of gestation, with an interquartile range of 34 + 1–36 + 3 weeks and range of 32 + 2–36 + 6 weeks. The study was approved by and followed the recommendations of the Regional Ethical Review Board in Gothenburg, Sweden (297-07, T675-10 and T510-12). Written and informed consent was obtained from the parents in accordance with the Declaration of Helsinki.

3 | RESULTS

The study population was born at a median of 34 + 5 weeks of gestation, with an interquartile range of 34 + 1–36 + 3 weeks and range of 32 + 2–36 + 6 weeks. The study was approved by and followed the recommendations of the Regional Ethical Review Board in Gothenburg, Sweden (297-07, T675-10 and T510-12). Written and informed consent was obtained from the parents in accordance with the Declaration of Helsinki.

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and height SDS increased from birth to five years of age, then followed a stable path from 5-10 years of age. Group data show increasing waist circumference. However, WHtR was stable over time. At 10 years of age, seven boys had a WHtR of more than 0.5 (range 0.54–0.62), indicating increased visceral fat mass19 (Figure 1).

The median concentrations and distributions of DHEAS and androstenedione increased stepwise between 5 and 10 years of age (Table 3). Figure 2 shows the longitudinal and individual distributions of DHEAS and androstenedione concentrations in relation to age. The correlations between DHEAS and androstenedione were significant and consistent at 5 years ($r = 0.69$), 6 years ($r = 0.70$), 7 years ($r = 0.63$), 8 years ($r = 0.67$) and 10 years of age ($r = 0.67$), $p < 0.001$ at all ages. No clinical signs of premature adrenarche were observed at eight years of age, but three boys had reached pubarche at 10 years of age.

No significant correlations were found between DHEAS or androstenedione at any age and gestational age or birth size. At the nominal level of significance, androstenedione at 5 years of age was, however, negatively correlated with birth length SDS ($r = −0.28$, $p = 0.05$).
Figure 3 illustrates the correlations between DHEAS and androstenedione with weight SDS and WHtR. DHEAS was significantly correlated with weight SDS from seven years of age and with WHtR at seven and 10 years of age. From seven years of age, androstenedione was significantly correlated with weight SDS, but not with WHtR at any age.

The median blood glucose at the start of the oral glucose tolerance test was 5.4 mmol/L with an interquartile range of 4.8–5.6 mmol/L and range of 3.3–6.9 mmol/L. The median post-load blood glucose at 120 min was 5.9 mmol/L with an interquartile range of 5.1–6.4 mmol/L and range of 3.3–8.3 mmol/L. Two boys had impaired glucose tolerance at 5 years of age, with post-load blood glucose of 8.2 and 8.3 mmol/L. No significant correlations were found between the oral glucose tolerance test at 5 years of age and birth data. In addition, the adrenal androgen data at 5 and 10 years of age were not correlated with the oral glucose tolerance test at 5 years of age.

In a longitudinal analysis, DHEAS and androstenedione concentrations were significantly associated with weight SDS and WHtR SDS, independent of age (Table 4).

4 | DISCUSSION

In this longitudinal study of boys born moderate to late preterm, we show for the first time that the individual trajectories of adrenal androgen secretion were consistent from 5–10 years of age. From seven years of age, androstenedione was significantly correlated with weight SDS, but not with WHtR at any age.

The foetal adrenal glands develop faster during the last 6 weeks of gestation, followed by rapid involution after birth.20,21 The dynamics of the adrenal gland and the set point of the hypothalamic-pituitary-adrenal axis may be influenced by premature birth.3 We have previously described how DHEAS and androstenedione in cord blood correlated with gestational age and how DHEAS also correlated with birth weight and length.11 This study shows that the correlation between DHEAS and current weight was still present...
during childhood, but we did not find any significant correlation between adrenal androgens and weight standard deviation score (SDS) and waist-to-height ratio (WHtR) in preterm boys. Cross-sectional analysis of correlations between dehydroepiandrosterone sulphate (DHEAS) and androstenedione and weight SDS and WHtR, respectively. Each coloured dot represents an individual value, and the different colours represent the different ages. Correlation coefficients (r) and p-values are denoted for 5–10 years of age. The dotted line represents the cut-off of 0.50. A p-value < 0.05 was considered significant.

**TABLE 4** Univariable and multivariable regression analyses of age and anthropometric variables and hormone levels

| Predictive variables | DHEAS (μmol/L) (univariable) | DHEAS (μmol/L) (adjusted for age) | Androstenedione (nmol/L) (univariable) | Androstenedione (nmol/L) (adjusted for age) |
|----------------------|-------------------------------|---------------------------------|---------------------------------------|---------------------------------------------|
| Age (years)          | β = 0.28                      | β = 0.09                        | β = 0.09                              | β = 0.09                                    |
|                      | p < 0.0001                    | p < 0.0001                      | p < 0.0001                            | p < 0.0001                                  |
| Weight (SDS)         | β = 0.38                      | β = 0.22                        | β = 0.08                              | β = 0.04                                    |
|                      | p < 0.0001                    | p < 0.0001                      | p < 0.0001                            | p < 0.0001                                  |
| WHtR (SDS)           | β = 0.45                      | β = 0.19                        | β = 0.10                              | β = 0.04                                    |
|                      | p < 0.0001                    | p < 0.0001                      | p < 0.0001                            | p < 0.0001                                  |

Note: Regression coefficients (β) from a linear mixed-effect model indicate the degree of change in each hormone level (units) for a one-unit change in the predictor. Age, weight SDS and WHtR SDS were the predictive variables for DHEAS and androstenedione concentrations at 5–8 and 10 years of age.

In adults, a WHtR cut-off of 0.50 indicates excessive fat and implies cardiovascular health risks. In children, WHtR is expected to decline during preschool years, along with changing body proportions. It has been suggested that a WHtR cut-off of 0.50 is also appropriate for children. However, because younger boys naturally have higher mean ratios at lower ages, there is a risk of overestimating WHtR as a risk factor in this group. In the present study’s longitudinal design, it was notable that the subjects who exceeded a WHtR of 0.50 at 5 years of age continued to show increasing WHtR, although we had expected this to decline with increasing age. Other studies have reported that children with catch-up growth after being born SGA had more visceral fat than children with normal birth sizes, even though they were not overweight. In addition, visceral fat accumulation was associated with increased DHEAS secretion. In the present study, we observed a greater weight gain in this cohort compared to normal reference intervals with an average weight gain.
of 0.5 SDS from birth to 5 years of age and 1.2 SDS to 10 years of age. These data indicate that changes in body composition occurred gradually from early childhood in this cohort.

Both DHEAS and androstenedione increased with age, and the levels seen in this study were consistent with previous studies of healthy boys. One of the major findings of this study was the robust course of the individual DHEAS secretion. Androstenedione did not show the same stringent trajectory as DHEAS before eight years of age, probably due to the higher inter-assay coefficient of variation at low concentrations. The results agreed with Liimatta et al., who found that DHEAS at one year of age correlated strongly with DHEAS at 6 years of age, indicating that the path of adrenal androgen secretion had already been established during infancy. The correlations between adrenal androgens and anthropometric parameters emphasise that weight gain and current body weight are important factors with regard to adrenal androgen synthesis during childhood. This statement is supported by one of the major findings of this study. This was that the longitudinal analysis showed that both DHEAS and androstenedione concentrations during childhood were related to weight and WHtR from 5-10 years of age, independent of age. These findings support studies that found that weight gain was an important factor that was also associated with adrenal androgen secretion in children with normal birth sizes. A previous study found that obesity affected DHEAS concentrations during childhood per se, even in children with normal birth weights. However, higher DHEAS concentrations was not reported in all obese children, but in those with central fat distribution indicated by a higher body fat percentage and higher waist circumference. Furthermore, even non-obese children with elevated DHEAS concentrations at seven years of age had a higher body fat percentage and higher waist circumference. The findings of this study, and others, emphasise the important and consistent association between body composition and adrenal androgen secretion.

4.1 | Strengths and limitations

The study strengths included the prospective design and using mass spectrometry-based method for hormone analysis. They also included the longitudinal analysis of adrenal androgen secretion patterns and the anthropometric measures and restricting the analysis to one gender. Some substantial limitations were the lack of a control group, the small cohort and the number of dropouts, which might have influenced the outcomes.

5 | CONCLUSION

This study of boys who were born moderate to late preterm showed that the trajectories of adrenal androgen secretion were consistent from 5-10 years of age, especially for DHEAS. Our data also showed that the weight and WHtR, rather than gestational age or birth size, were correlated with adrenal androgen secretion.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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