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Growth and adrenarche: findings from the CATS observational study

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ABSTRACT

Background There is increasing evidence that patterns of pubertal maturation are associated with different patterns of health risk. This study aimed to explore the associations between anthropometric measures and salivary androgen concentrations in pre-adolescent children.

Methods We analysed a stratified random sample (N=1151) of pupils aged 8–9 years old from 43 primary schools in Melbourne, Australia from the Childhood to Adolescence Transition Study. Saliva samples were assayed for dehydroepiandrosterone (DHEA), DHEA-sulfate and testosterone. Anthropometric measures included height, weight, body mass index (BMI) and waist circumference. Associations between (1) anthropometric measures and each androgen, and (2) hormone status with obesity and parental report of pubertal development were investigated using linear regression modelling with general estimating equations.

Results Greater height, weight, BMI and waist circumference were positively associated with higher androgen concentrations, after adjusting for sex and socioeconomic status. Being overweight or obese was associated with higher testosterone and DHEA concentrations compared with the normal BMI category. Those who were obese were more likely (OR=2.7, 95% CI 1.61 to 4.43, p<0.001) to be in the top tertile of age-adjusted androgen status in both sexes.

Conclusion This study provides clear evidence for an association between obesity and higher androgen levels in mid-childhood. The adrenal transition may be a critical time period for weight management intervention strategies in order to manage the risk for metabolic problems in later life for high-risk individuals.

What is already known on this topic?

- Studies examining associations between adrenarche and body composition or obesity have reported conflicting findings.
- There have been variable findings regarding the presence or absence of sex differences in the relationship between adrenarche and body composition or obesity.

What this study adds?

- Using a population-based sample, this study reports clear associations between anthropometric markers including body mass index and waist circumference and raised androgen levels in mid-childhood.
- This association is independent of age, with no evidence of difference between sexes.

INTRODUCTION

Transitioning through puberty is associated with the emergence of physical and mental health problems, including those related to obesity and metabolic syndrome.[1 2] Patterns of adrenal maturation are associated with different patterns of health risk, with early or accelerated adrenarche associated with features of metabolic syndrome.[3] Maturation of the adrenal cortex in childhood is thought to be triggered by key hormones including adrenocorticotrophic hormone, growth hormone, insulin-like growth factors, insulin and leptin.[4] This maturation results in increasing serum concentrations of adrenal androgen hormones dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEA-S) from approximately 6 to 8 years of age.[4 5] In peripheral tissues, DHEA and DHEA-S are metabolised into the androgens testosterone and dihydrotestosterone, which contribute to body hair growth and adult body odour in both sexes. The increase in circulating levels of DHEA and DHEA-S precedes the physical manifestations of adrenarche, and thus many children will exhibit increasing hormone levels associated with adrenarche before displaying any phenotypic signs.[6]

Studies investigating associations between body composition and the later phase of puberty, gonadarche, mostly support an association between earlier pubertal timing and greater adiposity during adolescence[7] and adulthood.[8] However, the few studies examining associations between adrenarche and body composition and obesity report conflicting findings.[1 9–12] While a number of studies have reported higher DHEA-S concentrations in obese compared with lean females[9] and males,[1 12] another study showed no cross-sectional association, but a longitudinal association between increasing DHEA-S levels and increasing body mass index (BMI),[11] and a further study reported no correlation between androgen levels and BMI.[10] Evidence for an association between pubic hair development and BMI is also conflicting, with studies reporting positive associations,[13 14] negative associations,[15] no relationship[16 17] and sex-specific relationships.[18]

Using a large cross-sectional dataset of peri-adrenarcheal children aged 8–9 years old from the Childhood to Adolescence Transition Study (CATS[19]), the primary aim of this epidemiological

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study was to explore associations between anthropometric measures and salivary androgen concentrations. The CATS Study has collected data from a large sample of participants in a narrow age range who represent a community sample from Melbourne, Australia, and as such is an excellent dataset to explore this association. Based on the postulated role of hormones associated with growth and adiposity in the initiation and progression of adrenarche,[4 20] we hypothesised a positive relationship between height, weight, waist circumference and BMI with androgen concentrations. We predicted that higher androgen levels would be associated with increased likelihood of expression of pubertal signs. Different findings have been reported regarding whether adrenarcheal timing and hormonal changes are sex related,[4 6] and in this study we sought to ascertain whether sex differences in levels of adrenarcheal hormones were present, suggestive of sex differences in adrenarcheal timing.

METHODS

Study design and participants

Our cross-sectional sample was taken from the first wave of the CATS Study; full eligibility criteria and all outcome variables are available in the published protocol.[19] The hormonal data from the first wave of the CATS Study have been published previously in studies examining body image dissatisfaction[21] and behavioural problems associated with adrenarche.[22] Participants were recruited from a stratified cluster sample of 43 primary schools in metropolitan Melbourne, Australia. All grade three students (fourth year of formal schooling) were invited to participate. Data collection took place over the course of the school year from April to October 2012. Of the 2289 children invited to participate, 1239 (54%) were recruited to the study.

Measures

Salivary androgen concentrations

Saliva samples were collected for DHEA, DHEA-S and testosterone assays. Salivary levels of unbound DHEA, DHEA-S and testosterone correlate highly with serum levels, and ultrasensitive immunoassays provide accurate, reproducible measurements.[23] Samples were collected in classroom settings between 09:00 and 10:00 using the passive drool method timed over 3 min. Students unable to provide 1 mL saliva were invited to provide a second sample in a small group setting (<2%). Samples were stored at −30°C and were analysed within 6 months of collection.

Sample volumes were measured before samples were centrifuged at 3000 RPM at 4°C for 15 min and assayed in duplicate using salivary enzyme immunoassay kits (Salimetrics, State College, Pennsylvania, USA; www.salimetrics.com), with the average concentration used for analyses. For DHEA, the range of sensitivity was 10.2–1000 pg/mL, the average intra-assay coefficient of variation (CoV) was 9.9% and the average interassay CoV was 16.5%. For testosterone, the range of sensitivity was 6.1–600 pg/mL, intra-assay CoV was 7.8% and interassay CoV was 13.2%. For DHEA-S, the range of sensitivity was 188.9–15 300 pg/mL, with 8.3% intra-assay and 9.1% interassay CoV. Salivary concentrations of DHEA-S are flow dependent,[24] so were adjusted for flow rate and displayed as pg/min. For androgen concentrations below the detectable limits, levels were estimated at half the lower detectable limit that is, 5.1 pg/mL for DHEA and 3.05 pg/mL for testosterone. No samples had undetectable DHEA-S concentrations. Since the androgen concentrations were expected to be positively skewed, they were log transformed before inclusion in regression models.

Anthropometric measures

Participant height, weight and waist circumference were measured by trained research assistants. BMI was calculated (kg/m^2). Height, weight and BMI were transformed to z-scores based on age and gender WHO 2007 growth standards.[25] BMI values were categorised into 'underweight', 'normal BMI', 'overweight' or 'obese' using the International Obesity Taskforce thresholds.[26] BMI was used as a measure of general adiposity, and waist circumference as an indicator of central obesity.

Pubertal development

Parents were invited to complete the Pubertal Development Scale (PDS) questionnaire assessing their children's pubertal development including: body hair growth, skin changes, breast development (girls) and menarcheal status (girls), voice changes (boys) and facial hair growth (boys).[27] Menarcheal status had a dichotomous (Yes/No) response. The remaining indicators had four possible responses: 'has not started yet'; 'has barely started'; 'has definitely started' and 'seems complete'. The PDS growth spurt question was excluded because it is not puberty specific. Based on questionnaire responses, participants were dichotomised into having clear puberty signs (if any 'has definitely started' or 'seems complete' response, or if a post-menarcheal female participant), versus having no clear puberty signs.

Covariates

To assess potential confounding, the following variables were included in regression models: child's age and sex, and family socioeconomic status (SES). Child's age in months at the time of the child assessment was used. Family SES was assigned using the Index of Relative Socio-economic Disadvantage scores from the Australian Bureau of Statistics census-based local neighbourhood Socio-Economic Index for Areas, categorised into quintiles for analysis.

Data analysis

Hormone measures, anthropometrics, puberty indicators, age and SES were summarised by sex (table 1). Mean and SD were calculated for continuous measures and percentages for categorical variables. Median and IQRs were calculated for androgen measures, as DHEA and DHEA-S distributions were positively skewed (see online supplemental figure S1). Pairwise correlation coefficients were calculated for raw and log-transformed androgen concentrations. Analyses were undertaken using Stata V.12 (Stata Corp, Texas, USA).

Investigating association between anthropometric and androgen measures

Linear regression models were used to estimate the individual effect of age and each anthropometric measure on each androgen concentration, with estimation using generalised estimating equations (GEE) with robust SEs to account for within-school clustering. Androgen concentrations were log transformed for the analyses. Age-adjusted and sex-adjusted z-scores were used for height, weight and BMI. BMI was also modelled categorically (underweight, normal BMI, overweight and obese) using the WHO standards, since in clinical and epidemiological practice and in terms of targeted interventions, BMI is usually treated as a categorical variable. Models were adjusted for age and SES as potential confounders, BMI z-score models were also adjusted for height. Each model was tested for a significant interaction between sex and

Table 1 Showing age, anthropometric, demographic, hormonal and pubertal status measures in children aged 8–9 years old (N=1151), stratified by sex; percentage of participants with valid data is included for each of the raw variables

	Girls		Boys	
Measure	**N (% total participants)**	**Mean (SD)**	**N (% total participants)**	**Mean (SD)**
Age (years)	628	8.95 (0.35)	523	8.98 (0.36)
Anthropometric measures				
Height (metres)	626 (>99)	1.34 (0.06)	522 (>99)	1.35 (0.06)
Weight (kg)	623 (>99)	32.3 (8.0)	522 (>99)	32.4 (7.2)
BMI	623	17.8 (3.2)	522	17.6 (3.0)
Waist circumference (cm)	623 (>99)	59.6 (8.0)	518 (>99)	60.1 (7.4)
Height (z-score)	626	0.29 (1.01)	522	0.45 (0.98)
Weight (z-score)	622	0.60 (1.16)	522	0.70 (1.16)
BMI (z-score)	623	0.58 (1.13)	522	0.60 (1.26)
SEIFA Disadvantage score	628	1012 (71)	523	1016 (65)
Salivary androgens				
Testosterone (pg/mL)	627 (>99)	22.1 (11.4)	517 (99)	19.6 (10.1)
DHEA (pg/mL)	627 (>99)	37.6 (36.2)	522 (>99)	27.8 (26.6)
DHEA-S (pg/mL)	617 (98)	1094 (1392)	513 (98)	1020 (1299)
Adjusted DHEA-S (pg/min)*	616 (98)	756 (1087)	513 (98)	788 (1298)
	N	**Median (IQR)**	**N**	**Median (IQR)**
Salivary androgens				
Testosterone (pg/mL)	627	20.5 (13.9)	517	18.8 (12.9)
DHEA (pg/mL)	627	26.8 (36.1)	522	18.8 (33.3)
DHEA-S (pg/mL)	617	667 (987)	513	632 (942)
Adjusted DHEA-S (pg/min)	616	427 (663)	513	422 (666)
	N (% total participants)	**n (% sample)**	**N (% total participants**	**n (% sample)**
BMI status	623		522	
Underweight		31 (5.0)		30 (5.7)
Normal BMI		431 (69.2)		381 (73.0)
Overweight		116 (18.6)		76 (14.6)
Obese		45 (7.2)		35 (6.7)
Pubertal development				
Changes in body hair	468 (75)	50 (10.7)	394 (75)	30 (7.6)
Changes in skin	462 (74)	22 (4.8)	394 (75)	5 (1.3)
Facial hair development		–	399 (76)	4 (1.0)
Voice deepening		–	398 (76)	7 (1.5)
Breast development	475 (76)	55 (11.6)		–
Periods commenced	470 (75)	7 (1.5)		–
Clear signs of puberty	478 (76)	91 (19.0)	401 (77)	31 (7.7)

Comparisons by sex were performed using t-tests for normally distributed data, Mann-Whitney-Wilcoxon tests for skewed data, and chi-X^2 tests for categorical variables.
*Adjusted DHEA-S (pg/min) was adjusted for salivary flow rate and was calculated using salivary weight (pg) and flow rate (min).
BMI, body mass index; DHEA, dehydroepiandrosterone; DHEA-S, DHEA-sulfate; SEIFA, Socio-Economic Index for Areas.

the independent anthropometric measure of interest in each model, as determined by $p<0.05$ in the sex×anthropometric measure variable in the model. The interaction did not meet this significance in any of the tested models (see table 2), so it was removed and both sexes were kept in the same model.

Investigating association between androgen levels with obesity and signs of puberty

We investigated whether exhibiting higher androgen concentrations relative to same-age peers (representing more advanced adrenarche) was related to obesity and pubertal stage. Relative androgen levels were estimated by calculating age-standardised residual androgen concentrations separately by sex and categorising these into low, moderate and high tertiles for each androgen. An overall androgen status index was constructed based on individuals' androgen tertile scores: those in the highest tertile for two or three androgens represented a high androgen group; those in the lowest tertile for two or three androgens represented a low androgen group; and the remainder represented an intermediate androgen group. We examined mean BMI z-score by tertile for each androgen and for overall androgen group, and used logistic regression models with GEE with a robust logit link to examine the association between androgen group and obesity. Cross-tabs were used to explore associations between androgen tertiles and overall androgen group and pubertal signs, stratified by sex, and logistic regression models with GEE with a robust logit link were used to model the association of each individual hormone tertile and androgen group with signs of puberty in girls and boys separately, accounting for within-school clustering and adjusting for age and SES.

RESULTS

A total of 1239 children were consented to participate in wave 1 of the CATS Study. This analysis focused on children aged 8–9 years, and excluded 88 participants outside this range (2

Table 2 Estimated associations between age, height (z-score), weight (z-score), BMI (z-score) and waist circumference and each of the salivary androgens testosterone, DHEA and DHEA-S, adjusted for sex and SES

	Testosterone			DHEA			DHEA-S		
	B	95% CI	P value	B	95% CI	P value	B	95% CI	P value
Age (years)	1.245	1.150 to 1.348	<0.001	1.362	1.178 to 1.576	<0.001	1.702	1.416 to 2.045	<0.001
Interaction with sex			0.3			0.6			0.3
Height z-score	1.116	1.082 to 1.151	<0.001	1.250	1.185 to 1.319	<0.001	1.267	1.196 to 1.344	<0.001
Interaction with sex			0.3			0.4			0.8
Weight z-score	1.134	1.103 to 1.167	<0.001	1.256	1.196 to 1.320	<0.001	1.216	1.154 to 1.282	<0.001
Interaction with sex			0.2			0.1			0.9
BMI z-score	1.090	1.053 to 1.129	<0.001	1.141	1.082 to 1.205	<0.001	1.142	1.076 to 1.212	<0.001
Interaction with sex			0.3			0.08			0.8
Waist circumference (cm)	1.012	1.007 to 1.018	<0.001	1.021	1.012 to 1.030	<0.001	1.011	1.001 to 1.022	0.04
Interaction with sex			0.2			0.2			0.4

The anthropometric analyses are adjusted for age, while BMI and waist circumference analyses are also adjusted for height. Each cell shows the coefficient for a partially adjusted model for the association of each androgen with age, height z-score, weight z-score, BMI z-score or waist circumference, respectively
Each model was tested for an interaction effect on hormone by sex. Results are presented as exponentiated coefficients (B; with 95% CI), so represent estimated geometric mean ratios per unit of the independent variable.
BMI, body mass index; DHEA, dehydroepiandrosterone; DHEA-S, DHEA-sulfate; SES, socioeconomic status.

participants <8 years, 86 participants ≥10 years) leaving 1151 participants. Salivary androgen data were available for >98% of participants. See table 1 for participant details including androgen, anthropometric and pubertal data.

Girls had higher salivary testosterone and DHEA concentrations than boys, while DHEA-S did not vary with sex. All three androgens were moderately correlated (untransformed correlation coefficients: r=0.30–0.66; log-transformed correlation coefficients: r=0.41–0.66). A total of 23.8% participants were overweight or obese, with no sex difference in underweight, overweight or obesity prevalence (table 1). Parent-reported pubertal data were available for 76.4% of participants (N=879; 478 girls). There were no group-wise differences between participants who provided data on pubertal status and those who did not in gender, age, hormone tertile or androgen group as assessed using X^2 tests and t-tests. Participants who did not provide pubertal status data were excluded listwise from the analysis comparing hormone groups and signs of puberty. Girls were more likely to have ≥1 clear pubertal sign than boys ($X^2(1879)=23.3$, $p<0.001$).

There were positive associations between age and androgen concentration, while there was no interaction between sex and age. table 2 shows regression estimates of age and anthropometric variables on androgen levels. Greater height, weight, BMI and waist circumference were positively associated with higher androgen concentrations after adjusting for sex, age and SES, with no sex interaction. Associations of BMI category with each androgen are shown in table 3. Being overweight or obese was associated with higher testosterone and DHEA concentrations compared with normal BMI. Obesity (but not overweight) was associated with higher DHEA-S concentrations. There were no differences in androgen concentrations between underweight and normal BMI groups.

Using androgen concentrations to assign children to androgen categories, 26.0% (n=300) were in the high androgen group, 45.9% (n=528) were in the intermediate androgen group and 28.0% (n=322) were in the low androgen group.

Mean BMI z-score rose across the tertiles for individual androgens and overall androgen status (see table 4). A total of 37.5% (102) of overweight or obese participants were in the high androgen group compared with 22.7% (184) of normal BMI children. Among obese children, 51.3% (41) were in the high androgen group compared with 24.1% (256) of non-obese children. These 102 obese or overweight children with high androgen status represent 8.9% of the total sample. Using logistic regression to model the association between androgen group and obesity, obese children were more likely to be in the high androgen group (OR 2.67 (1.61 to 4.43), $p<0.001$) compared with the intermediate androgen group, with no differences between the low and intermediate groups. Table 5 shows the proportion of children with clear pubertal signs in each androgen tertile and androgen group, stratified by sex. More girls than boys showed clear signs of puberty (X^2 (1879)=23.32, $p<0.001$). Girls with clear pubertal signs were more likely to be

Table 3 Estimated associations between BMI status and androgen concentration, showing the concentration of each hormone (with 95% CIs) for each of the BMI status groups

	Testosterone (N=1138)		DHEA (N=1143)		DHEA-S (N=1123)	
BMI status	Relative hormone concentration (95% CI)	P value	Relative hormone concentration (95% CI)	P value	Relative hormone concentration (95% CI)	P value
Underweight	0.97 (0.84 to 1.12)	0.7	0.84 (0.64 to 1.10)	0.2	0.95 (0.66 to 1.36)	0.8
Normal BMI	1		1		1	
Overweight	1.27 (1.17 to 1.37)	<0.0001	1.43 (1.24 to 1.65)	<0.0001	1.16 (0.95 to 1.41)	0.15
Obese	1.39 (1.22 to 1.58)	<0.0001	1.74 (1.37 to 2.22)	<0.0001	1.76 (1.32 to 2.35)	<0.0001
Interaction by sex		*0.18*		*0.05*		*0.5*

The reference group is normal BMI. Models were adjusted for age, sex and SES.
BMI, body mass index; DHEA, dehydroepiandrosterone; DHEA-S, DHEA-sulfate; SES, socioeconomic status.

Table 4 Mean BMI z-score (with 95% CI of the mean) for each hormone tertile and androgen group

	BMI z-score
Individual hormone tertile	**Mean (95% CI)**
Testosterone (n=1138)	
Low tertile	0.31 (0.19 to 0.41)
Intermediate tertile	0.49 (0.37 to 0.61)
High tertile	0.97 (0.83 to 1.12)
DHEA (n=1143)	
Low tertile	0.32 (0.18 to 0.46)
Intermediate tertile	0.56 (0.43 to 0.68)
High tertile	0.89 (0.76 to 1.02)
DHEA-S (n=1123)	
Low tertile	0.37 (0.26 to 0.49)
Intermediate tertile	0.73 (0.61 to 0.82)
High tertile	0.69 (0.55 to 0.84)
Androgen group (n=1139)	
Low	0.31 (0.16 to 0.43)
Intermediate	0.52 (0.44 to 0.60)
High	1.01 (0.85 to 1.18)

BMI, body mass index; DHEA, dehydroepiandrosterone; DHEA-S, DHEA-sulfate.

in the high testosterone (OR 2.12 (1.20 to 3.75), p=0.01) and DHEA tertile (OR 1.65 (1.05 to 2.57), p=0.03) than the intermediate tertile. Boys with clear pubertal signs were more likely to be in the high DHEA-S tertile than the intermediate tertile (OR 2.32 (1.22 to 4.44), p=0.01). For both boys and girls, individuals in the low androgen group were less likely to exhibit clear pubertal signs than those in the intermediate androgen group (girls OR 0.41 (0.22 to 0.76), p=0.005; boys OR 0.39 (0.17 to 0.90), p=0.03).

Table 5 Showing the proportion of participants with clear signs of puberty in each hormone tertile and androgen group, stratified by sex

	Clear signs of puberty	
	% (n)	
Individual hormone tertile	**Male**	**Female**
Testosterone		
Low tertile	8.5 (12)	11.4 (18)
Intermediate tertile	7.9 (10)	18.1 (28)
High tertile	7.1 (9)	27.4 (45)
Total	7.9 (31)	19.1 (91)
DHEA		
Low tertile	5.2 (7)	11.6 (18)
Intermediate tertile	8.8 (12)	18.1 (29)
High tertile	9.4 (12)	27.2 (44)
Total	7.8 (31)	19.1 (91)
DHEA-S		
Low tertile	3.7 (5)	11.7 (19)
Intermediate tertile	6.1 (8)	19.6 (28)
High tertile	13.6 (17)	25.5 (42)
Total	7.7 (30)	18.9 (89)
Androgen group		
Low	4.3 (5)	9.2 (12)
Intermediate	10.1 (18)	20.4 (46)
High	7.6 (8)	27.3 (33)
Total	7.7 (31)	19.1 (91)

DHEA, dehydroepiandrosterone; DHEA-S, DHEA-sulfate.

DISCUSSION

In this paper, we describe the results of a large cross-sectional population-based study of salivary adrenal androgens and anthropometry measures in Australian children during adrenarche. The key finding was a strong association between anthropometric markers of body composition and salivary adrenal androgen concentration, with consistent associations between greater height, weight, BMI and waist circumference and adrenal androgens, independent of age, for both sexes. Children with overweight or obesity had higher androgen concentrations. In secondary analyses, children with obesity were 2.7-fold more likely to be in the high androgen group, reflecting more advanced adrenarche. The 9% of participants who were overweight or obese and in the high androgen group could represent a group with potentially high future cardiometabolic risk. Previous studies have shown that children with premature adrenarche and raised DHEA-S have raised cardiometabolic risk markers,[28–30] but that this association may be explained by raised BMI, emphasising that this group of young people may be particularly suitable for targeted interventions to manage weight gain and obesity. A follow-up study of the Finnish cohort[29] reported that young women (aged 16.5–23.5 years) who had experienced premature adrenarche continued to show evidence of insulin resistance and had a tendency towards central fat mass distribution, but that cardiometabolic risk factors were no longer associated with adrenarcheal timing (after accounting for BMI across the study population).[31] Further studies examining the long-term impact of adrenarcheal timing and androgen concentrations in late childhood are needed to further explore the trajectories of these risks, including studies of the impact of the normal variability associated with adrenarche as opposed to those diagnosed with premature adrenarche, for whom there are very few data.

We found positive relationships between height, weight, BMI, waist circumference and obesity with salivary androgen concentration during adrenarche, in keeping with many previous studies using serum samples[1 32] although others report no association.[10] Conflicting findings may relate to sample differences including recruitment, age, prevalence of obesity, sample size and methodology of sampling androgen concentration. Most previous studies have focused on serum DHEA-S concentration and on female samples, with fewer published data evaluating associations between body composition in adrenarche and DHEA or testosterone, particularly in males.

The timing of biochemical adrenarche (ie, rising levels of DHEA-S) in girls has been shown to be highly heritable (0.61±0.09).[33] While genome-wide association studies have identified gene loci which may be associated with both pubertal timing and obesity and cardiometabolic risks,[34 35] no similar work has been published for adrenarche, and the potential for genetic associations between adrenarcheal timing and obesity or cardiometabolic risks has yet to be explored. The strong associations between anthropometric measures and androgen concentrations may reflect indirect and direct effects of growth and adiposity on androgen levels. Greater adiposity has been related to earlier signalling of adrenal maturation, increased bioactivation of circulating androgen levels, and accelerated adrenarcheal progression[36 37] through mechanisms involving leptin, insulin, growth hormone and insulin-like growth factors. Leptin has multiple effects including promoting adrenal androgen formation, synchronising the luteinising hormone-releasing hormone pulse generator, and triggering pituitary, adrenal and gonadal maturation.[37 38] The growth hormone/insulin-like growth factor 1 system, involved in growth signalling, has been linked to the enhancement of enzymes involved in androgen production.[39]

Children with insulin resistance and high circulating insulin concentrations can exhibit premature adrenarche and a predisposition to higher BMI,[40 41] although not all studies replicate this.[42] Insulin has also been demonstrated to stimulate adrenal androgen secretion, resulting in correlating insulin and testosterone levels in obese children.[43] Given recent trends for increasing levels of childhood overweight and obesity, the association between anthropometric measures and androgen levels may lead to increased incidence of early or premature adrenarche and long-term changes in growth and health.

A number of studies have focused on the links between premature adrenarche, defined as physical signs of adrenarche in girls <8 years or boys <9 years, and health outcomes. Premature adrenarche has been linked to hyperinsulinaemia and dyslipidaemia during puberty, adolescent presentations of polycystic ovarian syndrome,[44] and increased risk of metabolic syndrome with its significant comorbidity in adulthood,[3] although another recent study did not replicate this.[31] In our study, few participants reported phenotypic pubertal changes despite elevated androgen levels. Hormonal adrenarcheal changes can significantly precede physical changes, and early physical signs can be subtle and difficult to identify. More girls reported signs of puberty than boys, and hormone concentrations were differentially associated with pubertal signs in boys and girls. This may reflect differential exposure to androgens, differences in levels of enzyme activity or sensitivity to adrenal hormones in peripheral tissues, or differences in sensitivity to and detection of the phenotypic changes associated with adrenarche.[6 18 45 46] Females undergo gonadarche at a younger age than males, and the pubertal signs reported in this study may reflect adrenarcheal changes (eg, body hair) or changes associated with other aspects of puberty for example, thelarche (breast development). Many studies have focused on the health risks associated with premature adrenarche, but our understanding of the significance of biochemical adrenarche (ie, rises in circulating hormone levels without overt physical signs) and the factors driving the relationship between biochemical and physical adrenarcheal timing remains limited. Using a large, community-based population, this study emphasises that there is an association between body composition and circulating levels of adrenarcheal hormones, even in the absence of perceived physical markers of adrenarche.

These analyses using cross-sectional data cannot determine direction of causality, and there may be bidirectional relationships between body composition and adrenarche. Androgens regulate longitudinal bone growth by stimulating epiphysial growth plates and enhancing growth factor response,[47] while increased circulating testosterone levels have been linked to increased adiposity and insulin resistance.[48] Bidirectional effects between adiposity and androgen levels may be particularly concerning, since children with overweight would enter adrenarche earlier and then risk gaining more weight during adrenarche. Identification of these high-risk children may be critical for potential weight management interventions to avoid long-term health consequences. These data represent the baseline of a longitudinal cohort and future analyses could investigate within-subject and between-subject change through development.

This study used data from a large population-based sample with a narrow age range focused on the peri-adrenarchal period with similar male and female participant numbers. As a stratified cohort sample, it is representative of children from across Melbourne, Australia. Salivary androgens and anthropometric indices were assessed using high-quality methods in a classroom setting, and these data were nearly complete (>98%). One study limitation is that only one saliva sample was collected per participant, a decision based on the logistical requirements involved in collecting and transporting samples. While androgens have significant diurnal variation in older populations, available data for children and young adolescents suggest that concentrations are relatively stable in this population.[49 50] By standardising the time at which the samples were collected, sampling was restricted to the expected diurnal peak time of androgen production. Future studies might consider alternative methods for analysing hormone concentrations to immunoassays including liquid chromatography tandem mass spectrometry methods (LC-MS/MS). LC-MS/MS has the potential to allow for even greater sensitivity and specificity of measurements than immunoassay, which may be advantageous in younger participants where hormone levels are low in order to detect the smallest rises in hormone concentrations. However, data for paediatric and for saliva-based samples are still relatively lacking to date, and there are significant additional resources required in terms of analytical equipment and technical skills.[51] Data for pubertal signs, collected using paper or online parental report, were incomplete (response rate was 76.5%), and our associations should therefore be interpreted with caution. Physical assessment of Tanner stage was not practical for this large-scale epidemiological study. However, parental report of pubertal development has been shown to be highly correlated with physician pubertal assessment in children ≤12 years,[52–54] particularly when used to group participants by relative pubertal status as opposed to assign them to an exact stage and is potentially more accurate measure than self-assessment.[53]

CONCLUSION

This study describes the variation in salivary androgen concentration for a large representative metropolitan Australian population of children during adrenarche. It provides clear evidence for an association between markers of body composition and obesity and salivary androgen concentrations. Early adrenarche needs further longitudinal study as a marker for high metabolic risk individuals, as the adrenal transition may represent a critical time period for weight management intervention strategies to manage the risk of obesity-related metabolic problems in adulthood.

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