Received: 7 May 2019 | Revised: 18 May 2020 | Accepted: 18 May 2020

DOI: 10.1111/ijpo.12685

ORIGINAL RESEARCH

Pediatric OBESITY WILEY

Contrasting impact of androgens on male and female adiposity, fat distribution and insulin resistance in childhood and adolescence (EarlyBird 75)

Jade Chynoweth | Joanne Hosking | Alison Jeffery | Jonathan Pinkney

Faculty of Health, University of Plymouth, Plymouth, Devon, UK

Correspondence
Miss Jade Chynoweth, University of Plymouth, Faculty of Health, Medical Statistics. N15, Plymouth Science Park, 1 Davy Road, Plymouth PL6 8BX, UK.
Email: jade.chynoweth@plymouth.ac.uk

Summary

Objectives: To investigate associations between androgens (testosterone, dehydroepiandrosterone sulphate [DHEAS] and androstenedione), adiposity, fat distribution and insulin resistance (IR) during childhood and adolescence.

Methods: Three hundred and seven children (170 [55.4%] boys; 137 [44.6%] girls) recruited at age 5 and studied annually until age 16: androgens (liquid chromatography tandem-mass spectrometry), anthropometry, body composition (dual-energy x-ray absorptiometry) and IR (homeostasis model assessment).

Results: Early adiposity was associated with earlier detection of androstenedione in both sexes, and DHEAS in boys. At puberty, higher androgen levels were associated with favourable metabolic changes in boys, but adverse metabolic effects in girls. In boys, higher free testosterone (FT) was associated with lower body fat and android/gynoid fat ratio (AGR) (both $P < .001$), but in girls higher total testosterone was associated with higher AGR. In girls only, higher androstenedione ($P = .02$) and FT ($P = .01$) was associated with higher IR during puberty.

Conclusions: In pre-pubertal children, adiposity is associated with higher secretion of androgen precursors. After pubertal onset, higher testosterone is associated with lower adiposity and AGR in boys, but higher AGR and IR in girls. Therefore, androgens have modest sex-specific associations with children's total body fat, fat distribution and IR.

KEYWORDS

androgens, insulin resistance, puberty, testosterone

1 | INTRODUCTION

Differences in concentrations of androgen precursors (AP) and testosterone in children may have significant long-term implications for adiposity, fat distribution and insulin resistance (IR). However, most previous research has been undertaken in children with adrenal disorders or obesity, whereas healthy children have been less well represented.

Before puberty, androgens are derived from adrenal AP, whereas gonadal androgen production dominates after the onset of puberty. The detection of AP, such as dehydroepiandrosterone sulphate (DHEAS) and androstenedione, indicates adrenocortical activation

(adrenarche),[1] and there is some evidence from small and selective studies that early adrenarche may be associated with IR and subsequent risks of type 2 diabetes and polycystic ovary syndrome.[2,3] Furthermore, AP also have been associated with obesity in pre-pubertal children.[4,5,6,7,8]

Androgen concentrations change with age and development and differ by sex. Indirect evidence suggests that gonadal androgen production is also influenced by adiposity. Thus, increasing obesity has been linked to declining age of puberty,[9] and earlier menarche associated with adult obesity.[10] Although early androgen production might be associated with IR, obesity and long term metabolic risks,[11,12] the implications of AP and testosterone for fat distribution and IR in healthy children without adrenal disorders have received little attention.

Therefore, the aim of this longitudinal study was to investigate whether AP and testosterone are associated with adiposity, fat distribution and IR over time, in a healthy cohort of mainly normal weight children without adrenal disorders. Four specific objectives were to investigate: (a) The effect of adiposity on the onset of AP secretion; (b) The effect of puberty and adiposity on androgen concentrations over time; (c) The effect of puberty and androgen concentrations on body fat and fat distribution over time; (d) The effect of androgen concentrations on IR over time.

2 | METHODS

2.1 | The EarlyBird cohort

The EarlyBird study is an observational study investigating childhood origins of diabetes and obesity. The present analysis focuses on the potential role of androgens. Ethical approval was granted by Plymouth Local Research Ethics Committee in 1999 and updated regularly. The study was conducted in accordance with the Declaration of Helsinki and International Committee on Harmonisation of Good Clinical Practice. Written consent from parents and assent from children were obtained at each visit. The methodology has been described.[13] Three hundred and seven healthy children (170 boys) age 5 were recruited from schools, sampled from high and low socioeconomic areas and followed up every year until age 16 (for a total of 12 consecutive years). The exclusion criteria among the children included: existing diabetes, pathological states likely to affect growth or body composition, moderate or severe physical disability, and long-term use of oral steroids. Another 40 children were subsequently recruited at age 9 and followed up every year until age 16 (for a total of 8 consecutive years), to compensate for withdrawals and balance sexes. Data were collected annually from age 5 to 16 starting in the year 2000. There was 80% retention of the cohort at age 16. Comparing characteristics at age 5 for the 78% of the original cohort who were followed up at age 16 to the 22% that were lost to follow up, showed no differences between the two groups in body mass index (BMI) SD scores (SDS), index of multiple deprivation (IMD) or height SDS in either sex.

2.2 | Clinical methods

Children attended after an overnight fast from 22.00 hours, and venesection was performed at 09.00 hours. Ethnicity and socio-demographics at age 5 (IMD 2004, free school meal entitlement of the child, working hours, occupation, education level and income of the parents) and medical history were recorded by a research nurse within a hospital department of child health. Height was measured to the nearest 1 mm in blind duplicate (Leicester Height Measure, Child Growth Foundation, London, UK) and weight to the nearest 100 g (Tanita bio-impedance scales, Tanita UK Ltd) every 6 months. Age-adjusted BMI SDS were calculated with reference to 1990 UK standards.[14] Age at peak height velocity (APHV) was used as the primary measure of puberty, determined as tangential velocity at the middle time-point of three consecutive height measurements recorded 6 months apart. Tanner stage was self-reported annually using simple illustrations.[15] Early menarche was defined as first menses <11 years.[16]

Total body fat was determined by Dual-energy X-ray Absorptiometry (DEXA) using the Prodigy Advance fan beam densitometer (GE Healthcare, Chicago, IL) and fat distribution was expressed as the android/gynoid fat ratio (AGR). Physical activity was measured annually from age 5 by accelerometry (ActiGraph [formerly MTI/CSA].[17,18,19] Children wore accelerometers for seven consecutive days at each follow-up, and only recordings capturing at least four days were accepted. Time spent in moderate-to-vigorous physical activity (MVPA) per day was recorded as minutes spent in activity equivalent to three metabolic equivalents (METs), that is, approximately 2500 ActiGraph counts/min.[20] The mean MVPA for each child (from age 9 to 16) was reported.

2.3 | Laboratory methods

DHEAS, androstenedione, and total testosterone (TT) were measured in serum by liquid chromatography tandem-mass spectrometry (LC-MS/MS) using the Waters Acquity Ultrahigh Performance Liquid Chromatography System and Quattro Premier Tandem Quadrupole Mass Spectrometry (Waters Corporation, MA). Assay CVs were <15%. Free testosterone (FT) was calculated using the formula of Vermeulen.[21] All children at visit ages 5 to 8, 268 children at visit age 9 and 71 children at visit age 10 had luteinizing hormone (LH) measured by sandwich immunoassay. LH was measured by sandwich immunoassay with the Siemens Centaur system (lower limit of detection 0.5 IU/L). From April 2005, LH was measured by electrochemiluminescent assay with the Roche E170 system (lower limit of detection 0.1 IU/L). Insulin was assayed by chemiluminescent immunometric assay (DPC Immulite, Los Angeles, CA), in weekly batches of frozen serum. The inter-assay CV was 8.0% at 2.87 mU/L, lower detection limit 2.0 mU/L, and cross-reactivity with proinsulin <1%. IR was calculated by homeostasis model assessment (HOMA).[22]

2.4 | Statistical methods

Data were analysed with the statistical programming language R.[23] Boys and girls were modelled separately due to differences including the timing of puberty. Non-normal continuous outcome variables were logarithmically transformed if appropriate. A square root transformation was applied to DHEAS in girls. Androgen levels were considered "detectable" if individuals' concentrations were measurable for at least two consecutive years. Associations between age at detection of androgens and early adiposity (ages 5-7) were analysed with linear regression. Temporal relationships (from age 9) between androgen levels, body fat, fat distribution and IR were investigated with linear mixed effects models, using the lme4 package[24] in R. Imputation of missing data was not performed and any observations with incomplete data for the variables of interest were not included in the models. Individuals were not excluded if there were any missing time points. Associations between androgen levels, body composition and IR were examined with each of these variables in turn as the dependent variable. Mixed-effects models were constructed for boys and girls separately, with study visit (age) as repeat measure, individual identity codes as random effects and other covariates as fixed effects. For androgen levels as the dependent variable, covariates tested for inclusion were age, APHV and total body fat; for body fat/fat distribution as the dependent variable, covariates tested for inclusion were age, APHV and androgen SDS; for IR as the dependent variable, covariates tested for inclusion were age, APHV, height SDS, fat distribution and minutes of MVPA per day. APHV was also substituted with the age at self-reported Tanner stage 2, 3 and 4 (each in turn) for each of the longitudinal models to address any differences relating to the use of this single measure of puberty. Androgen levels were represented as SDS when included as covariates (because concentrations increased over time). Androgen SDS were standardised for sex and visit age group. The significance of candidate variables in linear regression and mixed-effects models were tested through forward and backward stepwise selection at 5% significance levels. Normality assumptions for linear and mixed effects models were checked visually using quantile-quantile plots. Outliers that influenced model assumptions (ie, data points that have a large effect on the slope of the regression line) were removed from analyses (if they did not affect the overall conclusion). Where interactions of factorial and continuous variables were modelled, effects such as age and BMI SDS were interpreted graphically by choosing approximately the 25th, 50th and 75th percentiles of the continuous variable and plotting the predicted outcome against the time factor for these reference levels.

3 | RESULTS

There were 3439 (1795 [52.2%] boys; 1644 [47.8%] girls) observations available between ages 5 and 16. 98% of the cohort were Caucasian with five children of mixed race. At age 5, the mean (95% confidence interval) IMD score in boys was 25.1 (23.0, 27.2) compared to 27.4 (24.8, 30.0) in girls (Table 1). At age 5, the mean (95% confidence interval) BMI SDS in boys was 0.19 (0.03, 0.35) compared to 0.53 (0.37, 0.69) in girls (Table 1). There were 2116 (1073 [50.7%] boys; 1043 [49.3%] girls) observations with DEXA data available between ages 9 and 16—in every model at least 70% of these observations were maintained.

3.1 | The effect of adiposity on the onset of androgen precursors and testosterone

The mean (95% confidence interval) age at androstenedione detection was 7.2 (6.9, 7.5) and 8.0 (7.6, 8.4) years in girls and boys respectively (Table 1). Androstenedione levels increased over time and by age 16 were higher in girls than boys (mean [95% confidence interval] 121.1 [110.9, 131.4] vs 84.4 [80.1, 88.6] nmol/l; $P < .001$) (Figure 1A). Mean (95% confidence interval) age at DHEAS detection was approximately 8 years in both sexes (Table 1)—girls 7.7 (7.4, 8.1) vs boys 7.7 (7.4, 8.0) and by age 16, concentrations were marginally higher in boys than girls (mean [95% confidence interval] 140.6 [129.8, 151.4] vs 125.2 [114.2, 136.3] nmol/L; $P = .052$) (Figure 1B). LH was detected in both sexes at approximately 11 years—girls 11.3 (11.1, 11.4) vs boys 11.3 (11.1, 11.5). TT was undetectable before 9 years, and became detectable at approximately 12 years (Figure 1C, D) in both sexes (Table 1)—girls 12.2 (11.9, 12.5) vs boys 12.1 (11.9, 12.3). Ages at androgen detection are summarised in supplementary file 1. Six girls had early menarche (first menses <11 years[16]). Three children had undetectable TT at 16 years (2 girls, 1 boy).

3.1.1 | Androstenedione

In boys, androstenedione detection age was weakly associated with BMI SDS at 6 years ($r = -0.15$, $P = .07$). The association was significant at 7 years ($r = -0.20$, $P = .009$), when a one-unit higher BMI SDS was associated with 5.7% (1.5%, 9.8%) or 8 months earlier androstenedione detection by age 12. In girls, BMI SDS at 5-7 years was also associated with age at androstenedione detection (r = [−0.19, −0.19, −0.22]; P = [.054, .047, .016]). For example, a one-unit higher BMI SDS at age 6 ($r = -0.19$, $P = .047$) was associated with 4.8% (0.1%, 9.3%) or approximately 7-month earlier androstenedione detection by age 12.

3.1.2 | Dehydroepiandrosterone sulphate

Higher BMI SDS at 5-7 years was associated with earlier DHEAS detection in boys (r = [−0.21, −0.21, −0.23]; P = [.03, .03, .01]) but not girls (r = [−0.15, −0.12, −0.09]; P = [.24, .21, .32]). In boys, a one-unit higher BMI SDS at age 5 ($r = -0.21$, $P = .03$) was associated with 4.0% (0.4%, 7.5%) or just under 6 months earlier DHEAS detection by age 12.

TABLE 1 Characteristics of the original cohort at age 5

	Boys (N = 170)	Girls (N = 137)	*P*-value
Age at baseline, y			
N (%)	170 (100)	137 (100)	.27
Mean (SD)	4.93 (0.26)	4.90 (0.25)	
Range	(4.37, 5.98)	(4.45, 5.97)	
BMI SDS at baseline			
N (%)	170 (100)	137 (100)	.004
Mean (SD)	0.19 (1.08)	0.53 (0.96)	
Range	(−2.37, 4.49)	(−2.54, 3.63)	
Overweight			
N (%)	16 (9.4)	17 (12.4)	.34
Obese			
N (%)	7 (4.1)	7 (5.1)	.68
Height SDS at baseline			
N (%)	168 (98.8)	137 (100)	.49
Mean (SD)	0.27 (1.08)	0.18 (1.06)	
Range	(−2.63, 3.43)	(−2.33, 3.06)	
IMD at baseline			
N (%)	167 (98.2)	131 (95.6)	.18
Mean (SD)	25.1 (14.0)	27.4 (15.4)	
Range	(5.0, 63.0)	(6.5, 73.2)	
Pubertal timing			
APHV, y			
N (%)	132 (77.6)	102 (74.5)	<.001
Mean (SD)	13.31 (0.83)	11.68 (1.12)	
Range	(10.63, 14.96)	(9.00, 14.67)	
Age at menarche, y			-
N (%)	N/A	102 (74.5)	
Mean (SD)		12.67 (0.99)	
Range		(10.41, 14.85)	
Age at first detection			
AD			
N (%)	148 (87.1)	124 (90.5)	<.001
Mean (SD)	8.01 (2.30)	7.19 (1.75)	
Range	(4.45, 14.52)	(4.59, 11.79)	
DHEAS			.67
N (%)	150 (88.2)	120 (87.6)	
Mean (SD)	7.67 (1.92)	7.74 (1.82)	
Range	(4.57, 12.75)	(4.59, 11.99)	
Total testosterone			.69
N (%)	137 (80.6)	102 (74.5)	
Mean (SD)	12.10 (1.26)	12.20 (1.50)	
Range	(9.32, 16.29)	(9.00, 16.72)	
LH			.67
N (%)	142 (83.5)	106 (77.4)	
Mean (SD)	11.30 (1.04)	11.25 (0.98)	
Range	(9.32, 14.04)	(9.00, 14.17)	

Abbreviations: AD, androstenedione; APHV, age at peak height velocity; DHEAS, dehydroepiandrosterone sulphate; LH, luteinizing hormone; IMD, index of multiple deprivation; SDS, SD score.

3.1.3 | Total testosterone

Detection age was investigated using TT. In boys, between 5 and 7 years, there were no associations between BMI SDS and TT detection age (r = [0.002, 0.03, −0.05]; P = [.44, .44, .99]). In girls, there was a borderline association at 5 years (r = −0.13, P = .06) but none at 6-7 years (r = [−0.07, −0.04]; P = [.17, .29]). However, in girls BMI SDS at 5-7 years was associated with earlier detection of LH (r = [−0.25, −0.27, −0.29]; P = [.006, .005, .003]). A one-unit higher BMI SDS at 5 years (r = −0.25, P = .006) was associated with approximately (∼) 3-months (∼1.5, ∼4.5) earlier LH detection. In boys, BMI SDS was not associated with age at LH detection.

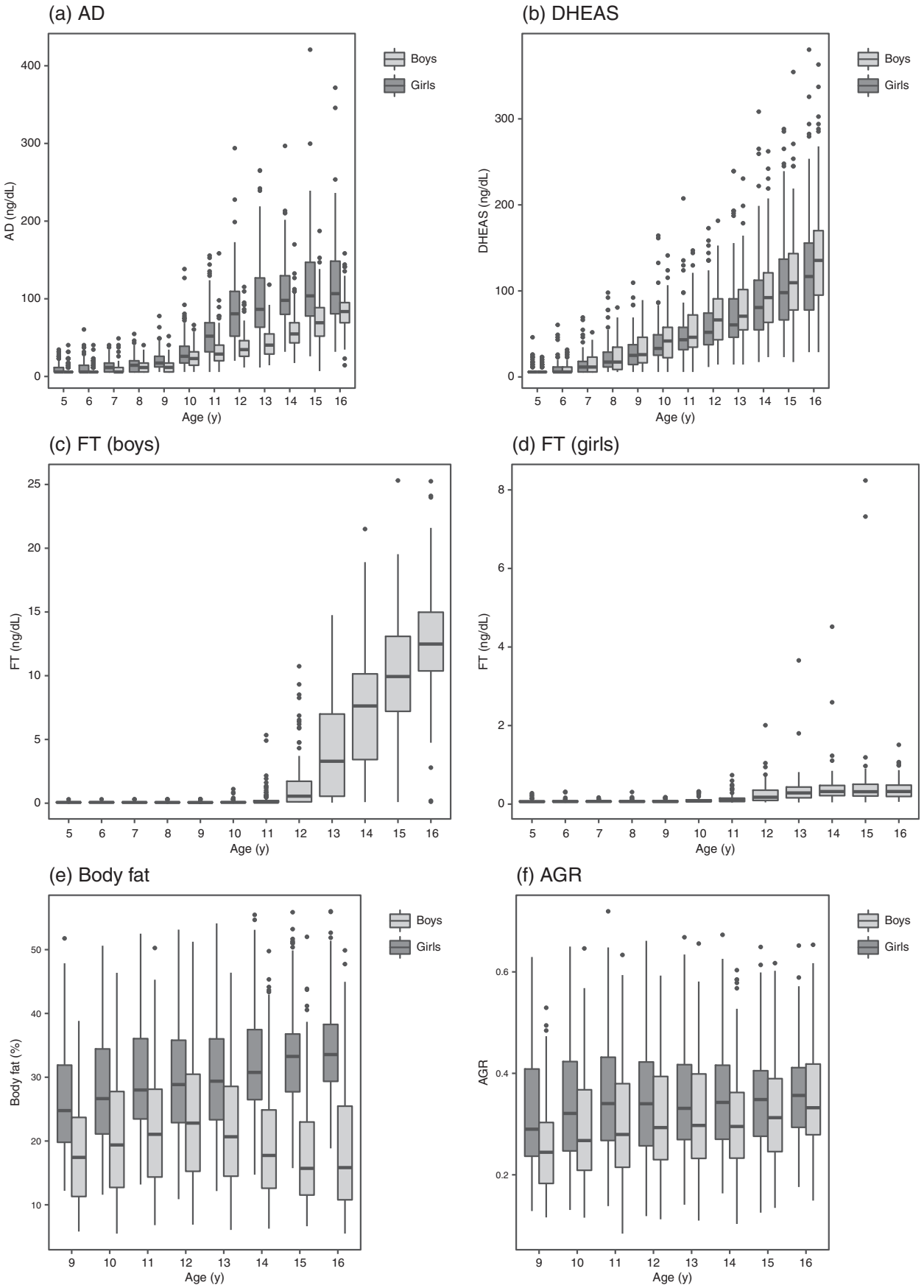

FIGURE 1 Boxplots of androgen precusors and free testosterone (separated by sex) for ages 5 to 16 years A-D; total body fat and android gynoid fat mass ratio (separated by sex) for ages 9 to 16 years E-F

TABLE 2 Longitudinal mixed effects modelling results

Boys Predictor	Androstenedione (n = 759)			DHEAS (n = 834)			Free testosterone (n = 776)		
	Coefficient	SE	*P*-value	Coefficient	SE	*P*-value	Coefficient	SE	*P*-value
Intercept	−2.86	1.43	.05	−10.92	3.96	.006	−179.20	16.02	<.001
age	0.62	0.11	<.001	2.11	0.21	<.001	30.05	2.60	<.001
age^2							−1.17	0.10	<.001
APHV	0.16	0.11	.14	0.59	0.30	.05	12.39	1.20	<.001
body fat	0.05	0.009	<.001				−0.01	0.004	.008
age: APHV	−0.02	0.008	.009	−0.09	0.02	<.001	−2.15	0.20	<.001
age^2: APHV							−0.09	0.008	<.001
age: body fat	−0.004	0.0007	<.001						
Girls Predictor	**Androstenedione (n = 794)**			**DHEAS (n = 802)**			**Free testosterone (n = 795)**		
	Coefficient	SE	*P*-value	Coefficient	SE	*P*-value	Coefficient	SE	*P*-value
Intercept	3.07	1.19	.01	−7.68	2.82	.007	−29.66	7.20	<.001
age	0.23	0.09	.01	1.67	0.17	<.001	4.92	1.17	<.001
age^2							−0.20	0.05	<.001
APHV	−0.35	0.10	<.001	0.39	0.24	.10	2.05	0.58	<.001
body fat	0.07	0.01	<.001	0.04	0.01	<.001	−0.13	0.08	.09
age: APHV	0.02	0.007	.03	−0.07	0.01	<.001	−0.39	0.09	<.001
age^2: APHV							0.02	0.004	<.001
age: body fat	−0.005	0.001	<.001				0.03	0.01	.04
age^2: body fat							−0.001	0.0005	.03

Note: Androstenedione and free testosterone was transformed with the natural logarithm in both sexes. DHEAS had a square root transformation applied in both sexes. Sample size is the number of observations available between ages 9 and 16.
Abbreviations: APHV, age at peak height velocity; DHEAS, dehydroepiandrosterone sulphate.

3.2 | The effect of puberty and adiposity on androgen concentrations over time

The primary analysis presented here and in Sections 3.3 and 3.4 was undertaken using APHV as the measure of puberty. The analysis was also undertaken using self-report Tanner stage as the measure of puberty. The findings were similar, and any differences are highlighted. The results are shown in Table 2. In both sexes, androstenedione concentrations were associated with age, earlier APHV and lower body fat by 16 years (Table 2). In boys, DHEAS concentrations were also associated with age and earlier APHV (Table 2). Two outliers (both age 15) were removed from the model due to their effect on the normality assumption and the results are shown in Table 2 (the findings remained significant with these outliers maintained in the model). In girls, DHEAS was associated with age, earlier APHV and higher body fat; for example, an increase in body fat from 25% to 35% in girls at age 12, with an average APHV of 12, was associated with approximately 5.7 ng/dL higher DHEAS (Table 2).

In both sexes, FT was associated with age, APHV, body fat and there were interaction effects with age for APHV and body fat. In boys, FT concentrations increased between 9 and 16 years, with a substantial increase in the rate of change by 13 years, and were associated with earlier APHV and lower body fat (Figure 2A, B). In girls, FT was associated with earlier APHV but higher body fat (Figure 2C, D). The findings above were consistent when age at self-reported Tanner stage 2, 3 or 4 was used as the measure of puberty but the interaction effect with age and body fat was no longer statistically significant in girls (P = .07; P = .08; P = .15 respectively).

3.3 | The effect of puberty and androgen concentrations on body fat and fat distribution over time

Results are shown in Figure 3.

3.3.1 | Androstenedione

Androstenedione SDS was unrelated to body fat (P = .20 boys; P = .99 girls). In boys, androstenedione SDS was unrelated to AGR (P = .11). In girls, however, AGR was predicted by age (P = .06), APHV (P = .14), androstenedione SDS (P = .001), and interaction effects of age with APHV (P = .02) and androstenedione SDS (P < .001). Androstenedione SDS was associated with lower AGR at age 9, but higher AGR by 16 years (Figure 3A).

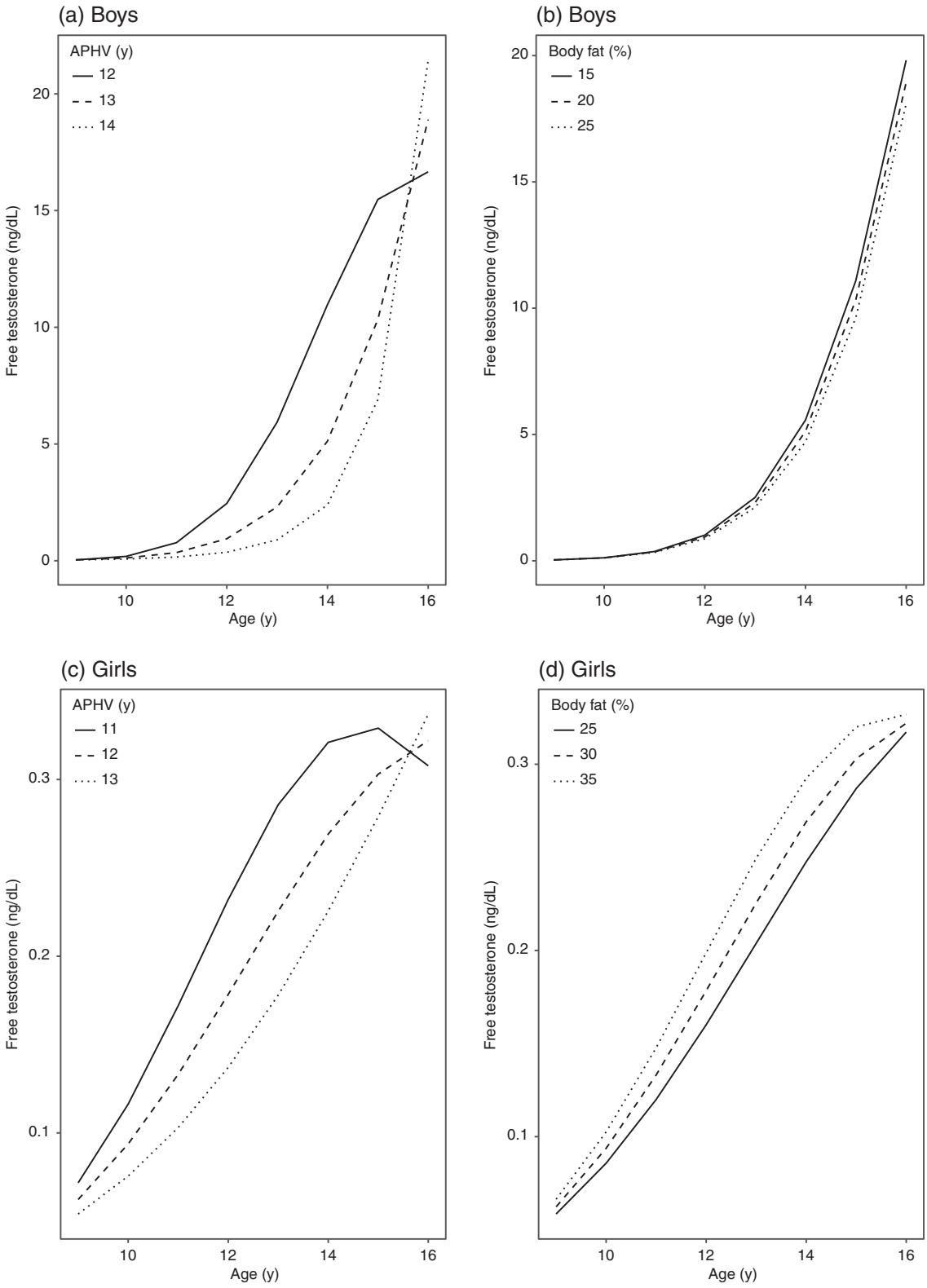

FIGURE 2 Interaction plots from longitudinal mixed effects models for age at peak height velocity (APHV) A and C, and total body fat B and D, when free testosterone concentration is an outcome for boys A and B, and girls C and D, separately between ages 9 and 16

3.3.2 | DHEAS

In boys, DHEAS SDS was unrelated to body fat (P = .18). In contrast, in girls, higher DHEAS SDS was associated with body fat. Body fat was predicted by age (P = .70), APHV (P = .07), DHEAS SDS (P < .001) and the interaction of age with APHV (P = .009). On average, a one-unit increase in DHEAS SDS was associated with approximately a 1% (0.44, 1.44) increase in body fat.

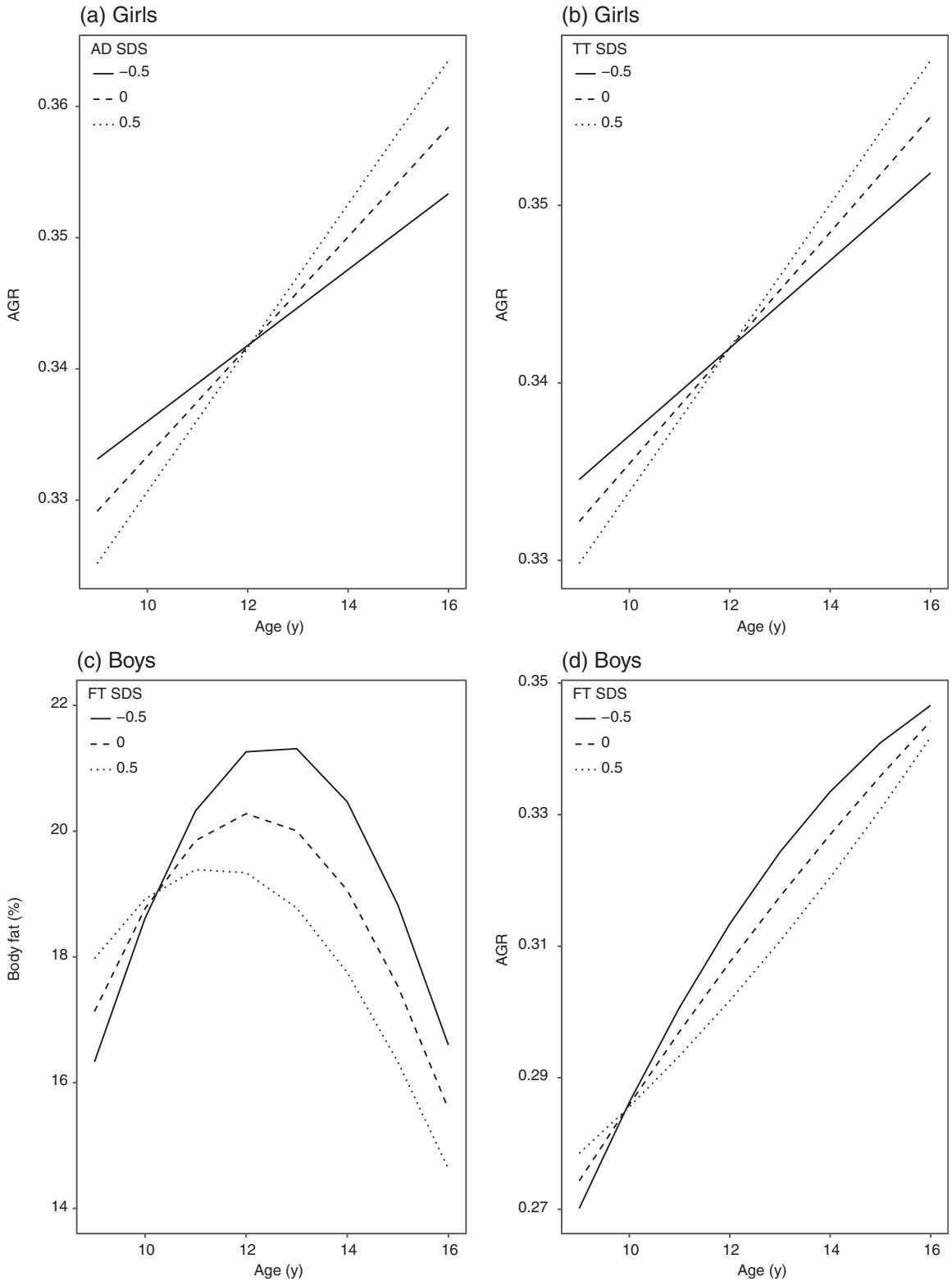

FIGURE 3 Interaction plots from longitudinal mixed effects models for androstenedione SD scores (SDS) A, total testosterone SDS B, and free testosterone SDS C and D, when android gynoid fat mass ratio A, B, D, or total body fat C, is an outcome for girls A and B, and boys C and D, separately between ages 9 and 16

In boys, DHEAS SDS was positively associated with AGR, with age, FT SDS and their interaction effect (see section on FT below) in the model. In contrast, DHEAS SDS in girls was not associated with AGR (P = .31).

3.3.3 | Free testosterone

In boys, from age 11, FT SDS was inversely associated with body fat (Figure 3C). Body fat was predicted by age, FT SDS and their

interaction (all $P < .001$). DHEAS SDS was not correlated with FT SDS so was therefore added into the model. AGR in boys was predicted by age ($P = .055$), DHEAS SDS ($P < .001$), FT SDS ($P = .001$) and the interaction between age and FT SDS ($P = .001$) (Figure 3D). In contrast, in girls, body fat had no association with FT SDS when age and APHV were included ($P = .098$). AGR was predicted by age and FT SDS (both $P < .001$). On average, a one unit increase in FT SDS was associated with an increase in android fat mass of 18 g/cm^2 (683 to 701 units when age = 12 and FT SDS increases from 0 to 1).

TT had similar effects to FT in both sexes for body fat and AGR in boys. The exception was that in girls from age 13, TT SDS was associated with higher AGR. AGR was predicted by age ($P < .001$), TT SDS ($P = .04$) and their interaction ($P = .03$) (Figure 3B).

3.4 | The effect of puberty, fat distribution, height, MVPA and androgen concentrations on IR over time

Before including androgens, IR in boys was predicted by age ($P < .001$), APHV ($P = .003$), height SDS ($P < .001$), AGR ($P < .001$), MVPA ($P = .04$), and the interaction of age with APHV ($P < .001$). Holding all other terms constant (age = 12, APHV = 12, height SDS = 0, MVPA = 20 minutes per day and an android fat mass of 600 g/cm^2 for a gynoid fat mass of 2000 g/cm^2 (ie, an AGR of 0.3): in boys, on average an increase in android fat mass of 200 g/cm^2 was associated with 24.4% increase in IR (0.93 to 1.16 IR units), and a one-unit increase in height SDS with 11.6% higher IR (0.93 to 1.04 IR units). Ten-minutes more MVPA per day was associated with 3.0% lower IR (0.93 to 0.90 IR units). At 10 and 14 years, IR was similar in boys regardless of APHV. However, at approximately 12 years, boys with earlier APHV exhibited higher IR. In contrast, by age 16, later APHV was associated with higher IR. Thus, at 12 and 16 years, 1-year increases in APHV were associated with 5.1% lower and 27.8% higher IR (0.93 to 0.88 IR units and 0.39 to 0.49 IR units). In girls, IR was predicted by age, height SDS and AGR (all $P < .001$). Keeping all other terms constant (as for boys), a one-unit increase in height SDS was associated with 25.1% higher IR (1.04 to 1.31 IR units), and a 200 g/cm^2 increase in android fat mass was associated with 24.7% higher IR (1.04 to 1.30 IR units).

3.4.1 | Androstenedione

Results are shown in Figure 4. In boys, there was no association between IR and androstenedione SDS ($P = .97$) (Figure 4A). In contrast, in girls, IR was predicted by age ($P < .001$), AGR ($P < .001$), height SDS ($P < .001$), androstenedione SDS ($P = .02$), the interaction of age with androstenedione SDS ($P = .008$), and the magnitude in the pubertal peak in IR was associated with higher androstenedione SDS (Figure 4B).

3.4.2 | DHEAS

There were no associations between IR and DHEAS SDS in boys or girls ($P = .52$ and $P = .50$ respectively).

3.4.3 | Testosterone

There was a marked sex difference in the relationship between FT and IR, and findings were similar with TT. In boys, there was no association between FT SDS ($P = .46$) and IR (Figure 4C). The findings above were consistent when age at self-reported Tanner stage was used as the measure of puberty. The interaction effect with age and FT SDS was not statistically significant when APHV was used as the measure of puberty but became statistically significant when Tanner stage 2-3 was used (both $P = .01$). In contrast, in girls, IR was predicted by age ($P < .001$), AGR ($P < .001$), height SDS ($P < .001$), FT SDS ($P = .01$) and the interaction of age with FT SDS ($P = .007$). In girls, the relationship between FT and IR changed over time, and the magnitude of the pubertal peak in IR at approximately age 12 was associated with higher FT SDS (Figure 4D). However, by age 16, the relationship between FT and IR in girls was not significant. When age at self-reported Tanner stage 4 was used as the measure of puberty, the interaction effect with age and FT SDS was no longer statistically significant in girls ($P = .12$).

4 | DISCUSSION

These data show that elevated androgen levels in childhood are associated with significant but small differences in adiposity, fat distribution and IR. These associations differ in girls and boys. In girls, the associations of higher androstenedione and testosterone with higher AGR and IR during puberty lie in the direction of increased cardiometabolic risk, whereas in boys higher testosterone was associated with lower AGR and no impact on IR. The contrasting relationship of androgens with body fat in boys and girls may be explained by differences in peripheral and central effects. The peripheral lipolytic effects of high testosterone concentrations in boys may reduce body fat. However, central effects may also be suggested by evidence that higher adiposity in boys is associated with later puberty[25] and relative hypogonadism.[26]

While it is thought that AP may influence fat distribution and IR, most previous work was undertaken in children with adrenal disorders, and so relevance to normal development is uncertain. Adrenarche is associated with higher expression of adrenocortical steroidogenic enzymes.[27] Recent research suggests this is a gradual process.[28] The absence of adrenarche in children with adrenocorticotropic hormone (ACTH) receptor mutations[29] and hypopituitarism[30] suggests that ACTH is required for adrenocortical activation. However, nutrition-related signals including insulin, IR, growth hormone/insulin-like growth factor 1 and leptin also influence adrenocortical development.[1] Consistent with this, higher infant adiposity and height were associated with premature adrenarche.[31] Therefore, nutritional influences may explain why premature adrenarche is associated with

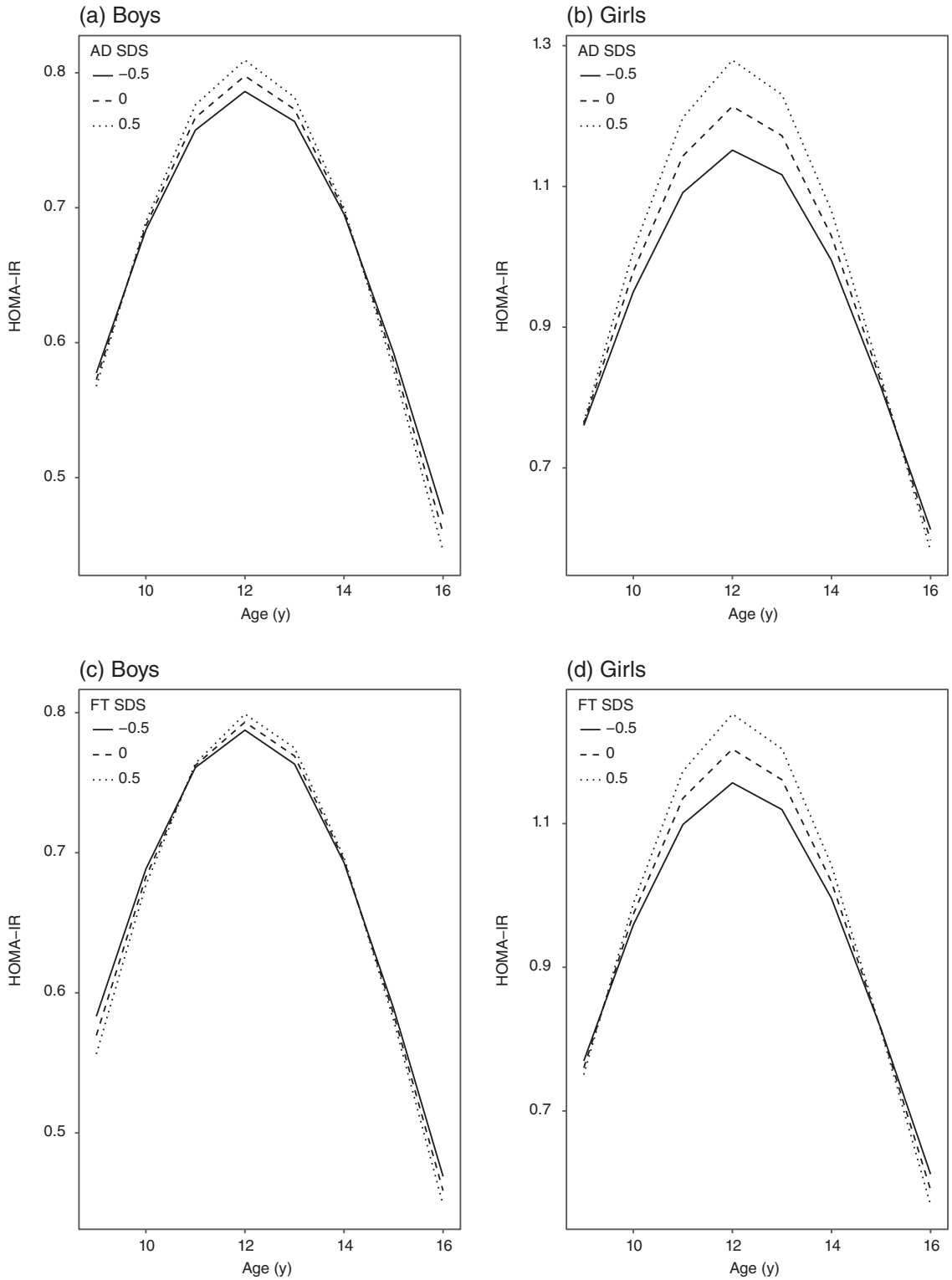

FIGURE 4 Interaction plots from longitudinal mixed effects models for androstenedione SD scores (SDS) A and B, and free testosterone SDS C and D, when insulin resistance (HOMA-IR) is an outcome for boys A and C, and girls B and D, separately between ages 9 and 16

higher weight and metabolic risks. The present results, in a cohort of healthy children, show that early adiposity (ages 5-7) is associated with higher AP production during normal development.

This study also demonstrated that higher concentrations of AP and testosterone were associated with earlier puberty. The findings were consistent with APHV or Tanner stage as the measure of puberty, with some sex differences. In girls, when age at self-reported Tanner stage 2, 3 or 4 was used instead of APHV (when modelling testosterone concentrations) the interaction of age with adiposity was no longer significant, suggesting that Tanner stage may be associated with testosterone concentrations, as might be expected. In boys, lower body fat was associated with testosterone concentrations,

whether APHV or Tanner stage was the measure of puberty. The findings with IR were consistent with APHV or Tanner stage as the measure of puberty in girls. The changes in IR in boys were explained by APHV and Tanner stage 4, but not Tanner stage 2 or 3. When Tanner stage 4 was used as the measure of puberty in girls, the interaction of age with testosterone was no longer associated with IR, suggesting that Tanner stage 4 may be associated with IR.

There are several reasons why AP might accelerate pubertal processes. In a previous study, higher early pubertal concentrations of androstenedione and estradiol were associated with faster pubertal development.[32] Although biologically inactive, AP are minimally bound by sex hormone-binding globulin,[33] and conversion to dihydrotestosterone or estradiol could be significant. Furthermore, adipose tissue metabolises sex steroids. For example, intracellular aromatisation of AP to estrone in adipose tissue[34] and subsequent conversion to estradiol[35] could associate adiposity with higher estradiol production. Adipose tissue can also generate and inactivate testosterone.[36] Therefore, not only can nutritional signals activate the adrenal cortex, but also adiposity amplifies and enhances sex steroid effects.

It is recognised that the present study has both strengths and limitations. The study builds on previous advances. The high sensitivity of isotope dilution gas chromatography mass spectrometry has advanced the study of androgens in children,[37] and is well suited to detecting low steroid concentrations. Androgen levels measured by LC-MS in children were reported by Meikle et al[38] and reference ranges reported elsewhere.[39,40,41] The findings of higher concentrations of DHEAS and testosterone in boys and androstenedione in girls, support previous observations.[41] The key strength of this study was the EarlyBird cohort itself, with high retention rate, and meticulous year-on-year measurements of growth, pubertal development, anthropometry, body composition, and endocrinology in contemporary British children.

The principal limitation is that while a cohort study can demonstrate association and temporal precedence, it does not prove causation. For example, the association of higher androgens with central adiposity during puberty in girls could have several explanations, including that obesity-related IR stimulates ovarian androgen production,[42] as well as the possibility that androgens might promote central adiposity in girls. Other potential limitations include the sample size (especially before age 11 when most children have undetectable testosterone), relatively lean population, and some children (particularly boys), had not completed puberty by age 16 when measurements stopped. Due to the lack of ethnic diversity in South West England, the cohort is limited to mostly white ethnic backgrounds therefore these data may not be generalisable to other ethnicities. There is also error in estimating age at detection of androgens and LH because of annual sampling. The less sensitive assay for LH was used until 2005, which limits the ability to determine the age at which LH was first detected in children with early puberty.

The analysis was strengthened by the availability of both APHV and self-reported Tanner stage reflecting growth hormone and sex steroid action spanning puberty. Tanner stage was self-reported to avoid repeated intimate physical examinations in adolescents. A benefit of Tanner stage is that it reflects a process over time, but drawbacks were its subjectivity, not being specific enough to differentiate between Tanner stages, sex difference and the variable stage reached by age 16 years. For example, only 197 out of 270 children (73%) reported at age 16 considered themselves to have reached Tanner stage 4, which substantially decreases the sample size for this measure. In contrast, APHV is a single time point but is an objective measure. It is noted that APHV occurs later in puberty for boys compared to girls, which is why girls and boys were modelled separately. The principal results of the study were the same, irrespective of whether puberty was measured by APHV or Tanner stage.

In conclusion, these data show that androgens contribute to the regulation of body weight and fat distribution in children. AP and testosterone have modest but significant effects on adiposity and fat distribution, with important sex contrasts. Higher androgen levels appeared to have potentially adverse effects on fat distribution and metabolism in girls and favourable effects in boys. However, it remains uncertain whether or not physiological differences in androgen concentrations in childhood lead to long-term differences of fat distribution and IR in adults. Longer-term studies may answer that question.

ACKNOWLEDGEMENTS

The EarlyBird study was the idea of our former colleague Professor Terry Wilkin (1945-2017), Professor of Endocrinology and Metabolism at the Peninsula College of Medicine and Dentistry in Plymouth. Terry designed and directed this study for many years. We gratefully acknowledge Terry's life work and key contributions to this paper. We also acknowledge the contribution made by Dr Linda Voss, study coordinator from 1999 to 2012, and other members of the EarlyBird team. The EarlyBird study was supported by the Bright Future Trust, the Kirby Laing Foundation, the Peninsula Foundation and the EarlyBird Diabetes Trust.

CONFLICT OF INTEREST

There are no conflicts of interest.

ORCID

Jade Chynoweth https://orcid.org/0000-0002-2516-5923

REFERENCES

1. Belgorosky Bacquedano MS, Gercio G, Rivarola MAA. Adrenarche: postnatal adrenal zonation and hormonal and metabolic regulation. *Horm Res*. 2008;70:257-267.
2. Ibanez Dimartino-Nardi J, Potau N, Saenger PL. Premature adrenarche: normal variant or forerunner of adult disease? *Endocr Rev*. 2000;21:671-696.
3. Utriainen Laakso S, Liimatta J, Jaaskelainen J, Voutilainen RP. Premature adrenarche—a common condition withb variable presentation. *Horm Res Paediatr*. 2015;83:221-231.
4. Remer Manz FT. Role of nutritional status in the regulation of adrenarche. *J Clin Endocrinol Metab*. 1999;84:3936-3944.
5. Corvalan Uuay R, Mericq VC. Obesity is positively associated with dehydroepiandrosterone sulfate concentrations 1 to 7 y in Chilean children of normal birth weight. *Am J Clin Nutr*. 2013;97:318-325.

6. Pintor Loche S, Faedda A, Fanni V, Nurchi AM, Corda RC. Adrenal androgens in obeses biys before nad after weight loss. *Horm Metab Res*. 1984;16:544-548.
7. Akyurek Atabek ME, Eklioglu BS, Alp HN. Is there a relationship between cardiovascualr risk factors and dehydroepiandrosterone sulfate levels in childhood obesity? *J Pediatr Endocrinol Metab*. 2015;28:545-550.
8. Mantyselka Jaaskelainen J, Lindi V, Viitasalo A, Tompuri T, Voutilainen R, Lakka TAA. The presentation of adrenarche is sexually dimorphic and modified by body adiposity. *J Clin Endocrinol Metab*. 2014;99:3889-3894.
9. Biro M, Greenspan LC, MPF G. Puberty in girls of the 21st century. *J Pediatr Adolesc Gynecol*. 2012;25:289-294.
10. Prentice Viner RMP. Pubertal timing and adult obesity and cardiometablic risk in woemn and men: a systematic review and meta-analysis. *Int J Obes*. 2013;37:1036-1043.
11. Kousta E. Premature adrenarche leads to polycystic ovary syndrome? Long term consequences. *Ann N Y Acad Sci*. 2006;1092:148-157.
12. Janghorbani Mansourian M, Hosseini EM. Systematic review and meta-analysis of age at menarche and risk of type 2 diabetes. *Acta Diabetol*. 2014;51:519-528.
13. Voss LD, Kirkby J, Metcalf BS, et al. Preventable factors in childhood that lead to insulin resistance, diabetes mellitus and the metabolic syndrome: the EarlyBird diabetes study 1. *J Pediatr Endocrinol Metab*. 2003;16(9):1211–1224. https://doi.org/10.1515/JPEM.2003.16.9.1211.
14. National Obesity Observatory. A simple guide to classifying body mass index in children. http://www.noo.org.uk/uploads/doc/vid_11601_A_simple_guide_to_classifying_BMI_in_children.pdf. Published 2011.
15. Taylor SJC, Whincup PH, Hindmarsh PC, Lampe F, Odoki K, Cook DG. Performance of a new pubertal self-assessment questionnaire: a preliminary study. *Paediatr Perinat Epidemiol*. 2001;15(1):88-94. https://doi.org/10.1046/j.1365-3016.2001.00317.x.
16. Gaudineau A, Ehlinger V, Vayssiere C, Jouret B, Arnaud C, Godeau E. Factors associated with early menarche: results from the French Health Behaviour in School-aged Children (HBSC) study. *BMC Public Health*. 2010;10:175.
17. Metcalf BS, Curnow JS, Evans C, Voss LD, Wilkin TJ. Technical reliability of the CSA activity monitor: the EarlyBird Study. *Med Sci Sport Exerc*. 2002;34(9):1533-1537. https://doi.org/10.1249/01.MSS.0000027715.99037.06.
18. Puyau MR, Adolph AL, Vohra FA, Butte NF. Validation and calibration of physical activity monitors in children. *Obes Res*. 2002;10(3):150-157. https://doi.org/10.1038/oby.2002.24.
19. Fox KRC. *Start Active, Stay Active: A Report on Physical Activity for Health from the Four Home Countries' Chief Medical Officers*. Richmond, Surrey, United Kingdom: The National Archives; 2011:16-19. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/216370/dh_128210.pdf. Accessed June 22, 2018.
20. Schmitz KH, Treuth M, Hannan P, et al. Predicting energy expenditure from accelerometry counts in adolescent girls. *Med Sci Sport Exerc*. 2005;37(1):155-161. https://www.ncbi.nlm.nih.gov/pubmed/15632682.
21. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab*. 1999;84(10):3666-3672. https://doi.org/10.1210/jcem.84.10.6079.
22. Matthews DR, Hosker JR, Rudenski AS, Naylor BA, Treacher DF, Turner RC. *Homeostasis Model Assessment: Insulin Resistance and Fl-Cell Function from Fasting Plasma Glucose and Insulin Concentrations in Man. Vol 28*. Berlin, Germany: Springer Science+Business Media; 1985. https://link.springer.com/content/pdf/10.1007/BF00280883.pdf. Accessed July 16, 2019.
23. R Core Team. R: A language and environment for statistical computing. https://www.r-project.org/. Published 2017.
24. Bates D, Bolker B, Walker S, Machler M. Fitting linear mixed-effects models using lme4. *J Stat Softw*. 2015;67(1):1-48. https://doi.org/10.18637/jss.v067.i01.
25. Vandewalle S, Taes Y, Fiers T, et al. Sex steroids in relation to sexual and skeletal maturation in obese male adolescents. *J Clin Endocrinol Metab*. 2014;99(8):2977-2985. https://doi.org/10.1210/jc.2014-1452.
26. Mushannen T, Cortez P, Stanford FC, Singhal V. Obesity and hypogonadism—a narrative review ing the need for high-quality data in adolescents. *Children (Basel, Switzerland)*. 2019;6(5):63. https://doi.org/10.3390/children6050063.
27. Nakamura Gang HX, Suzuki T, Sasano H, WEY R. Adrenal changes associted with adrenarche. *Rev Endocr Metab Disord*. 2009;10:19-26.
28. Auchus JR. The physiology and biochemistry of adrenarche. *Endocr Rev*. 2011;20:20-27.
29. Weber Clark AJ, Perry LA, Honour JW, MOA S. Diminished adrenal androgen secretion in familial glucocorticoid deficiency implicates a significant role for ACTH in the induction of adrenarche. *Clin Endocrinol*. 1997;46:431-437.
30. Boettcher Hartmann MF, de Lafolie J, Zimmer KP, SAC W. Absent adrenarche in children with hypopituitarism: a study based on urinary steroid metabolomics. *Horm Res Pediatr*. 2013;79:356-360.
31. Marakaki Karapanou O, Gryparis A, Hochberg Z, Chrousos G, Papadimitriou AC. Early adiposity rebound and premature adrenarche. *J Pediatr*. 2017;186:72-77.
32. de Ridder M, Thijssen JH, Bruning PF, van den JKL B, Zonderland ML, WBC E. Body fat mass, body fat distribution, and pubertal development: a longitudinal study of physical and hormonal sexual maturation of girls. *J Clin Endocrinol Metab*. 1992;75:442-446.
33. Dunn F, Nisula BC, Rodbard DJ. Transport of steroid hormones: binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. 1981;53:58-68.
34. Mendelson R, Simpson ERC. Regulation of estrogen biosynthesis by human adipose cells in vitro. *Mol Cell Endocrinol*. 1987;52:169-176.
35. MacDonald C, Madden JD, Brenner PF, Wilson JD, PKP S. Origin of estrogen in normal men and in women with testicular feminization. *J Clin Endocrinol Metab*. 1979;49:905-916.
36. O'Reilly MW, House PJ, Tomlinson JW. Understanding androgen action in adipose tissue. *J Steroid Biochem Mol Biol*. 2014;143:277-284.
37. Soeborg Fredericksen H, Fruekilde P, Johenssen TH, Juul A, AMT A. Serum concentrations of DHEA, DHEAS, 17α-hydroxyprogesterone, Δ4-androstenedione and testosterone in children determiend by TurboFlow-LC-MS/MS. *Clin Chim Acta*. 2013;419:95-101.
38. Meikle W, Kushnir MM, Rockwood AL, Pattison EG, Terry AH, Sandrock TA. Adrenal steroid concentrations in children seven to seventeen years of age. *J Pediatr Endocrinol Metab*. 2007;20:1281-1291.
39. Soldin P, Sharma H, Husted L, Soldin SJO. Pediatric reference intervals for aldosterone, 17α-hydroxyprogesterone, dehydroepiandrosterone, testosterone and 25-hydroxy vitamin D3 using tandem mass spectrometry. *Clin Biochem*. 2009;42:823-827.
40. Kushnir M, Blamires T, Rockwood AL, et al. Liquid chromatography—Tandem Mass Spectrometry assay for Androstenedione, Dehydroepiandrosterone, and Testosterone with pediatric and adult reference intervals. *Clin Chem*. 2010;56:1138-1147.
41. Soeborg Fredericksen H, Mouritsen A, Johannsen TH, et al. Sex, age, pubertal development and use of oral contraceptives in relation to serum concentrations of DHEA, DHEAS, 17α-hydroxyprogesterone, Δ4-androstenedione, testosterone and their ratios in children, adolescents and young adults. *Clin Chim Acta*. 2014;437:6-13.
42. Barbieri RL, Makris A, Randall RW, Daniels G, Kistner RW, Ryan KJ. Insulin Stimulates androgen accumulation in incubations of ovarian

stroma obtained from women with hyperandrogenism. *J Clin Endocrinol Metab*. 1986;62(5):904-910. https://doi.org/10.1210/jcem-62-5-904.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Chynoweth J, Hosking J, Jeffery A, Pinkney J. Contrasting impact of androgens on male and female adiposity, fat distribution and insulin resistance in childhood and adolescence (EarlyBird 75). *Pediatric Obesity*. 2020;e12685. https://doi.org/10.1111/ijpo.12685