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High DHEAS Level in Girls Is Associated with Earlier Pubertal Maturation and Mild Increase in Androgens throughout Puberty without Affecting Postmenarche Ovarian Morphology

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Keywords
Adrenarche · Early puberty · Hyperandrogenism · High dehydroepiandrosterone sulfate · Ovarian morphology

Abstract
Objective: To assess whether the presence of high DHEAS (HD) at 7 years determines different timing, sequence, and rate of pubertal events, and whether it is associated with adrenal and/or ovarian hyperandrogenism and changes in ovarian morphology throughout puberty. ***Methods:*** In a longitudinal study of 504 girls, clinical evaluation was performed every 6 months after 7 years of age to detect Tanner stages; hormonal and anthropometric measurements were conducted at thelarche (B2), breast Tanner 4 (B4), and 1 year after menarche; ultrasonographic evaluation was also performed after menarche. The girls were classified as HD if their DHEAS level was >42.1 µg/dL (>75th percentile) around 7 years. ***Results:*** HD around 7 years is associated with a younger age at thelarche, pubarche, and menarche. Girls with HD had higher androstenedione and total testosterone levels, and a higher free androgen index (FAI), and lower levels of antimüllerian hormone (AMH) at B2, and higher levels of androstenedione and FAI at B4 and after menarche. All these results were significant even after adjusting for body mass index, age at first DHEAS determination, and birth weight. One year after menarche, polycystic ovarian morphology was detected in 7.6 and 7.3% of the HD and the normal DHEAS group, respectively. Ovarian volume was correlated with AMH, testosterone, androstenedione, and LH but not with DHEAS around 7 years. ***Conclusion:*** Prepubertal HD in normal girls was associated with earlier thelarche, pubarche, and menarche, and a mild androgen increase throughout puberty. We believe continuous follow-up of this cohort is important to prospectively address the interrelationships between biochemical adrenarche and early growth as determinants of ovarian function.

Introduction

Adrenarche is the progressive maturational process of the adrenal zona reticularis resulting in increased secretion of the adrenal androgen precursor dehydroepian-

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drosterone (DHEA) and its sulfate ester (DHEAS) [1, 2]. Premature adrenarche (PA) is defined biochemically by concentrations of DHEAS above the prepubertal level before the age of 8 years in girls and 9 years in boys: 40 μg/dL (1.08 μmol/L) are within normal limits for early puberty but above average for children 6–8 years of age [3]. Adrenarche is clinically recognized by the presence of signs of androgen action, including adult-type body odor, oily skin, comedones/acne, and axillary and pubic hair growth.

PA has been associated with increased adiposity and metabolic risk [3, 4]. However, early infancy weight gain has also been associated with increased metabolic risk, earlier puberty, and PA [5–7]. We recently found that high DHEAS (HD) around 7 years was associated with an increased risk of precocious pubertal events only in girls independent of adiposity and birth weight [8].

Areas of controversy regarding the comorbidities associated with biochemical adrenarche include whether there is an impact on the timing and sequence of pubertal events, and whether HD around 7 years determines an altered pattern of ovarian and adrenal androgen concentrations during pubertal development and ovarian morphology once menarche has occurred. Previously described associations may depend on the prevalence of low birth weight and ethnic background of the study population.

We took advantage of a well-characterized cohort of children enrolled in the Growth and Obesity Chilean Cohort Study (GOCS), a longitudinal study of Chilean children that included anthropometric determinations, pubertal assessment, and the measurement of hormonal concentrations during puberty. The two aims of this study were: first, to assess whether the presence of HD around 7 years determines a different timing, sequence, and rate of pubertal events. Second, we determined whether HD is followed by an altered pattern of adrenal androgen concentrations, early ovarian hyperandrogenism, and changes in ovarian morphology.

Subjects and Methods

Subjects

We longitudinally evaluated 602 girls recruited in 2006 at the age of 3.0–4.9 years attending the Chilean National Nursery School Council Program (JUNJI) in the southeastern area of Santiago, Chile. The current study is a cross-sectional follow-up within GOCS, whose primary aim was to assess the association between early growth and development of adiposity and metabolic risk during childhood [9]. The initial inclusion criteria were as follows: singleton birth, gestational age 37–42 weeks, birth weight ≥2,500 and ≤4,500 g, and an absence of physical or psychological conditions that could severely affect growth [10].

Annual evaluations were conducted (anthropometry, body composition, skeletal and hormonal maturation, and metabolic/inflammatory markers), and, in 2009, we began to collect Tanner staging data on a 6-month basis. Maternal age at menarche was self-reported by the mother.

The Ethics Review Board of the Institute of Nutrition and Food Technology (INTA) of the University of Chile approved the study protocol. All parents or guardians of the children provided written informed consent, and the children gave their assent.

Pubertal Development

At the age of about 7 years, a single pediatric endocrinologist (V.M.) assessed pubertal staging via inspection and palpation according to the Tanner scale [11]. Subsequently, secondary sex characteristics were evaluated every 6 months by a single female dietitian trained specifically for this purpose, with permanent supervision of a single pediatric endocrinologist (V.M.). Breast Tanner stage concordance between the dietitian and the pediatric endocrinologist was 0.9 [12].

Age at menarche was self-reported. Girls were advised to call researchers from the first menses, and telephonic follow-up was performed every 6 months starting at breast Tanner stage 4 (B4); a questionnaire was developed to differentiate vaginal infections or other genitourinary conditions from the first menses.

Anthropometric Measurements

Weight and height were determined using standardized protocols (barefoot and light clothes) by 2 dietitians with inter- and intrarater correlation coefficients >0.80 for all measurements. Weight was measured with a portable electronic scale (Seca 770), with a precision of 0.1 kg, and height was measured with a portable stadiometer (Harpenden 603) to the nearest 0.1 cm. Body mass index (BMI) was calculated by dividing weight in kilograms (kg) by height in meters squared (m^2). We described height for age and BMI for age in standard deviation scores (SDS) based on the WHO 2006 standards and the WHO 2007 growth reference [13].

We defined obesity as BMI-SDS ≥2. A complete description of the anthropometric methodology is found elsewhere [14]. Birth weight was obtained from medical records, and the quality of the data was previously assessed [15].

Ultrasonographic Study

Gynecological ultrasound examinations were performed 1 year after menarche by 2 observers using a GE Logiq P 5 Ultrasound system (GE KPI Health Care Inc., CA, USA) with a 5-MHz transabdominal probe. The ultrasound was performed during the early follicular phase of the menstrual cycle (postmenstrual days 2–7). Measurements were performed in real time with the highest possible magnification to view the ovaries. The longest medial axis (length) and its corresponding thickness and width were measured to calculate ovarian volume (OV) according to the formula of the volume of a sphere or prolate ellipse (0.5 × length × width x thickness in cm) [16]. Polycystic ovarian morphology (PCOM) was defined as the presence of an OV >12 mL in at least 1 ovary according to the definition of PCOM in adolescents by the World Pediatric Consensus for Polycystic Ovarian Syndrome (PCOS) [17]. The intra-observer variation coefficients of the ultrasonographic study were 3.2 and 4.1% for

Table 1. Clinical and anthropometric characteristics of 504 girls by DHEAS levels at around 7 years of age

	High DHEAS $n = 137$ (27%)	Normal DHEAS $n = 367$ (73%)	p value
Age at DHEAS measurement, years	6.9±0.4	6.7±0.4	<0.001
Weight, kg	26.9±5.3	24.5±4.3	<0.001
SDS	1.0±1.1	0.6±1.0	<0.001
Height, cm	121.8±5.6	119.9±5.2	<0.001
SDS	0.3±1.0	0.2±0.9	0.087
BMI SDS	1.2±1.1	0.7±1.0	<0.001
Waist-to-hip ratio	61.4±7.1	58.1±6.1	<0.001
Weight change 0–2 years (>0.67 SDS), *n* (%)	31 (23%)	95 (26%)	0.489
Obesity (>2 SDS), *n* (%)	27 (20%)	44 (12%)	0.006
Birth weight, kg	3.3±0.4	3.4±0.4	0.022
SDS	0.2±0.8	0.3±0.8	0.056
Maternal age at menarche, years	12.6±1.6	12.8±1.7	0.322
Gestational age, weeks	39.2±1.2	39.1±1.3	0.387

SDS, standard deviation scores.

OV, respectively. In cases where a dominant follicle or cyst >10 mm was observed, the ultrasound was repeated during another menstrual cycle. Adolescents with pathological images in the ultrasound assessment were excluded from the analysis. In each patient, the ovary with the larger OV and larger number of follicles was reported. In total, 396 ultrasounds were performed, 26 measurements were repeated, and 1 girl was excluded because of pathological findings.

Hormonal Determinations

Hormonal levels were determined by a blood sample at around 7 years of age and during pubertal progression by a fasting venous sample obtained early in the morning.

Mothers were contacted the day before blood sampling to confirm the absence of fever (37.5 °C) or symptoms of acute infection in the children. Samples were analyzed at the Institute of Maternal and Child Research, University of Chile. At around 7 years of age, serum DHEAS was determined by competitive specific binding RIA as we previously described [8] supplied by Diagnostic System Laboratories (Webster, TX, USA); the intra- and interassay coefficients of variation were 3.5 and 5.1%, respectively. Thereafter, samples were obtained at breast Tanner stage 2 (B2), B4, and 1 year after menarche. The latter sample was obtained in the early follicular phase (days 2–7 of the menstrual cycle) before 8:30 a.m.

Concentrations of DHEAS, androstenedione17-OH progesterone (17-OHP), and testosterone were analyzed by liquid chromatography-mass spectrometry in a high-performance liquid chromatography (HPLC) Agilent system (Santa Clara, CA, USA) 1260 coupled to an AB Sciex 3200 Quantum ultratriple quadrupole mass spectrometer (Foster City, CA, USA).

The liquid chromatography separation was carried out on a 150-mm long column with 300 µm of internal diameter packed with 4 µm Synergi Hydro-RP particles and maintained at 40 °C. Samples were processed by a Chromsystems kit (Chromsystems Instruments & Chemicals, Gräfelfing, Germany). Samples, calibrators, and quality controls were run in duplicate and prepared according to the manufacturer's instructions. Briefly, 25 µL of precipitation reagent and 200 µL of internal standard were added to 100 µL of serum sample. After an incubation period of 10 min, samples were centrifuged at 15,000 *g* for 5 min, and 200 µL were transferred into the vials. Fifty microliters were injected into the HPLC-tandem mass spectrometry system. The total run time was 10.5 min. Steroid recovery was between 81 and 108%. The sensitivities for DHEAS, androstenedione, 17-OHP, and testosterone were 75, 0.03, 0.05, and 0.01 ng/mL, respectively. The corresponding intra-assay coefficients of variation were 2.9, 1.2, 2.0, and 2.9%, respectively. The corresponding interassay coefficients of variation were 5.0, 6.8, 2.5, and 2.8%, respectively. The measurements of luteinizing hormone (LH), follicle-stimulating hormone (FSH) (sensitivity = 0.06 mIU/mL), and sex hormone-binding globulin (SHBG) (sensitivity = 0.5 nmol/L) were performed using an immunoradiometric assay (Izotop Laboratories, Budapest, Hungary). Serum estradiol was measured by RIA (Pantex, Santa Mónica, CA, USA). The sensitivity of this assay is 5.0 pg/mL. Serum antimüllerian hormone (AMH) was measured using the Beckman-Coulter Gen 2 ELISA assay with no predilution (Immunotech; Beckman Coulter Inc., Prague, Czech Republic). The intra- and interassay coefficients of variation for FSH, LH, SHBG, estradiol, and AMH were 4.0–5.3, 4.5–5.6, 3.9–6.9, and 5.7–7.9% and both less than 5%, respectively. SHBG and testosterone were used to calculate the free androgen index (FAI), as reported previously [18].

Statistical Analysis

Girls were categorized into the HD group or the normal DHEAS (ND) group based on the sample obtained in 2009 at around 7 years of age. The girls were classified as HD if they had a DHEAS level >42.1 µg/dL (>75th percentile of the distribution of our population) [14].

Age at any pubertal event was considered as the midpoint between 2 consecutive visits, e.g., age at B2 was considered the age between the last visit at B1 and the visit in which B2 was detected. In the case that a girl was already at B2 or greater when we started Tanner evaluation (2009) ($n = 39$), we assumed that she was Tanner 1 at age 5 years (in 2006–2007, the girls did not have any signs of puberty).

Table 2. Pubertal timing, rate, and sequence of 504 girls participating in the Growth and Obesity Chilean Cohort Study according to DHEAS levels at around 7 years of age

	High DHEAS at 7 years $n = 137$ (27%)	Normal DHEAS at 7 years $n = 367$ (73%)	p value
Age at B2, years	$n = 127$ 8.6±1.6	$n = 345$ 9.3±1.3	<0.001
Age at B4, years	$n = 124$ 10.7±0.8	$n = 328$ 11.1±0.9	<0.001
Age at pubarche, years	$n = 133$ 9.3±1.1	$n = 349$ 9.7±0.9	<0.001
Age at menarche, years	$n = 128$ 11.6±1.0	$n = 361$ 12.1±1.0	<0.001
Time B2-menarche, years	$n = 120$ 2.9±1.3	$n = 343$ 2.8±1.1	0.211
BMI-SDS at B2	$n = 127$ 1.23±1.1	$n = 344$ 0.79±1.09	<0.001
BMI-SDS at B4	$n = 127$ 1.25±1.0	$n = 327$ 0.86±1.10	<0.001
BMI-SDS 1 year after menarche	$n = 103$ 1.30±1.0	$n = 272$ 0.89±1.04	<0.001
B2 before pubarche, *n* (%)	$n = 137$ 69 (50%)	$n = 367$ 178 (49%)	0.764

B2, breast Tanner 2; B4, breast Tanner 4; SDS, standard deviation score.

Descriptive analyses (mean, SDS, and percentage) of anthropometric, hormonal characteristics, and sexual maturation data were performed by stratifying by HD and ND status. Statistical differences between the groups (HD vs. ND) were assessed using the χ^2 and Student *t* test, as appropriate, and the Mann-Whitney test was used to compare differences in the medians of hormonal levels.

Linear regression models were performed to assess the relationship between DHEAS around 7 years of age and pubertal onset, timing and progression, hormonal profile during puberty, and ovarian morphology 1 year after menarche adjusting for chronological age at DHEAS sampling, BMI-SDS at age of DHEAS sampling, birth weight, and maternal age at menarche.

Analysis was carried out in STATA version 15.0, and the results were considered significant at a value of $p < 0.05$.

Results

In 2009, we collected blood samples from 504 girls (84% of the original cohort) who were evaluated when they were around 7 years old and classified the participants by DHEAS level into the HD and ND groups. HD was present in the blood samples of 137 girls (27%). The mean birth weight of the HD group was slightly lower than that of the ND group (Table 1). Girls with HD had a higher mean BMI at age 7 years, a higher waist-to-hip ratio, and a greater prevalence of obesity. The percentage of girls with fast weight gain, defined as a change between birth and 2 years of ≥0.67 SDS, was not different between the girls in the HD and ND groups (Table 1).

Timing, Sequence, and Rate of Pubertal Events

B2 was detected during biannual visits in 472 girls (HD, $n = 127$), and B4 was detected in 452 girls (HD, $n = 124$); 380 adolescents completed the follow-up 1 year after menarche (HD, $n = 105$). The girls with HD presented earlier B2, earlier pubarche (P2), earlier age at B4, and earlier menarche. There was no difference in the first clinical secondary sex characteristic detected, being thelarche in 50 and 49% of HD and ND girls, respectively. Nevertheless, the mean time difference between B2 and menarche was similar in the HD and ND girls (Table 2). In

linear regression analyses between DHEAS levels around 7 years of age and pubertal timing and ovarian volume, the above findings persisted even after adjusting for age at DHEAS sampling, BMI-SDS around 7 years, birth weight, and mother's age at menarche (online suppl. Table 1; for all online suppl. material, see www.karger.com/ doi/10.1159/000506632).

Hormonal Profile

Girls with HD around 7 years had higher concentrations of DHEAS; androstenedione, testosterone, and FAI at B2 than those with ND. The situation was similar at B4 and 1 year after menarche, except for testosterone, which did not differ between the groups after B2. Furthermore, a lower AMH level was observed at B2, and a lower SHBG level was observed from B4 onwards in the HD group. No difference between the HD and ND girls was detected in serum gonadotropin or estradiol concentrations during the follow-up (Table 3). In linear regression models, once the hormonal concentrations and FAI were adjusted by age and BMI SDS at first DHEAS determination around 7 years of age, BMI at pubertal stage, and birth weight SDS, HD was associated with higher androstenedione and testosterone at B2 and B4 and with higher FAI 1 year after menarche. Moreover, HD around 7 years was associated with lower AMH levels at B2 and with lower SHBG levels 1 year after menarche (online suppl. Table 2).

Ultrasonographic Assessment

The mean OV was similar in the HD and ND groups 1 year after menarche. PCOM was equally frequent in girls with HD versus ND (Table 3). OV correlated with AMH ($r = 0.24$, $p < 0.001$), LH ($r = 0.16$, $p < 0.005$), total testosterone ($r = 0.16$, $p < 0.005$), androstenedione ($r = 0.12$, $p < 0.05$), FAI ($r = 0.12$, $p < 0.05$) in all postmenarcheal adolescents, even after adjusting for potential confounders (BMI and birth weight). OV was not correlated with DHEAS levels at 7 years.

Table 3. Hormonal profile and ovarian morphology according to DHEAS levels at around 7 years of age and pubertal stage

	Breast Tanner 2			Breast Tanner 4			1 year after menarche		
	high DHEAS $n = 127$	normal DHEAS $n = 345$	p value	high DHEAS $n = 124$	normal DHEAS $n = 328$	p value	high DHEAS $n = 105$	normal DHEAS $n = 275$	p value
DHEAS, µg/dL	87 (64–113)	51 (36–67)	0.001	119 (89–149)	72 (52–95)	<0.001	124 (85–166)	72 (51–98)	<0.001
17-OHP, ng/mL	0.23 (0.15–0.32)	0.23 (0.16–0.35)	0.519	0.34 (0.25–0.53)	0.35 (0.25–0.50)	0.546	0.39 (0.29–0.56)	0.45 (0.32–0.65)	0.084
Androstenedione, ng/mL	0.28 (0.20–0.40)	0.23 (0.16–0.34)	0.005	0.77 (0.62–1.07)	0.71 (0.55–0.96)	0.032	0.96 (0.69–1.14)	0.81 (0.64–1.03)	0.028
Testosterone, ng/mL	0.07 (0.05–0.10)	0.06 (0.04–0.08)	0.008	0.18 (0.12–0.25)	0.16 (0.12–0.23)	0.099	0.20 (0.16–0.24)	0.18 (0.14–0.24)	0.147
AMH, ng/mL	3.15 (1.97–4.68)	4.07 (2.66–5.60)	0.002	1.86 (1.15–2.94)	2.19 (1.32–3.31)	0.070	2.94 (1.92–3.99)	3.03 (1.97–4.54)	0.631
LH, mIU/mL	0.29 (0.23–0.42)	0.31 (0.24–0.44)	0.705	3.5 (2.1–5.3)	3.0 (2.0–4.9)	0.144	3.2 (2.1–4.5)	3.4 (2.3–4.6)	0.086
FSH, mIU/mL	2.0 (1.3–3.3)	2.2 (1.5–3.4)	0.176	5.8 (4.4–7.0)	5.9 (4.7–6.9)	0.343	5.7 (4.6–6.9)	6.0 (5.0–7.2)	0.125
SHBG, nmol/L	60 (46–79)	63 (44–84)	0.568	37.8 (30.2–54.8)	45.6 (32.4–60.1)	0.020	32 (25–42)	41 (29–51)	<0.001
Estradiol, pg/mL	14 (10–22)	13 (9–19)	0.260	38 (21–54)	37 (20–53)	0.756	23.5 (18–33)	26.0 (19–35)	0.166
Free androgen index	0.37 (0.25–0.69)	0.28 (0.18–0.52)	0.021	1.54 (0.95–2.70)	1.23 (0.81–1.78)	0.004	2.04 (1.49–2.78)	1.57 (1.16–2.37)	<0.001
Ovarian volume							7.1 (5.5–9.2)	7.3 (5.7–9.1)	0.503
PCOM, n (%)							8 (7.6%)	20 (7.3%)	0.530

Values are presented as medians (interquartile ranges) unless indicated otherwise. PCOM, polycystic ovarian morphology.

Discussion

In this longitudinal sample of term-born girls with normal birth size recruited from the community, we observed that having high concentrations of DHEAS at 7 years was associated with earlier age at thelarche, pubarche, and menarche with persistent higher concentrations of androgens (although within the normal range) through puberty, even after adjusting for BMI-SDS and age at DHEAS stratification and birth weight. Higher

DHEAS levels at around 7 years of age were associated with lower AMH levels at the beginning of puberty. Finally, HD and ND adolescents had similar OV 1 year after menarche, and a similar percentage of PCOM prevalence. OV correlated positively with LH, AMH, testosterone, and androstenedione in all adolescents.

In 2014, in a study by Mäntyselkä et al. [19], the prevalence of PA, defined by a serum DHEAS concentration ≥1 μmol/L (≥37 μg/dL) and any clinical sign before the age of 8 years in girls and 9 years in boys, was 8.6% in girls and 1.8% in boys, despite a similar prevalence of biochemical adrenarche between the sexes. This cross-sectional study did not evaluate later associations of adrenarche. We previously reported, in the same cohort analyzed in this study, that HD was associated with a 2.6-times greater risk of precocious thelarche and a 3-times higher risk of precocious pubarche compared to ND girls [8]. Therefore, we decided to follow these girls to study whether the described clinical and biochemical associations described in Catalonian PA girls [20–23] persisted throughout pubertal development in our study subjects with higher DHEAS at the age of 7 years.

Previous studies have not been able to fully elucidate the relationship of biochemical PA (i.e., high DHEAS concentrations) with ovarian hyperandrogenism. Most of the studies included patients with clinically evident signs of adrenarche. Ibañez et al. [20, 22], in a group of 35 Catalonian adolescents with PA (premature pubarche as a clinical sign), reported that 45% of the adolescents developed PCOS characterized by hirsutism, menstrual disturbances, and elevated androgen levels. Ovarian stimulation with a GnRH analog in this cohort resulted in exaggerated levels of 17-OHP and androstenedione, which correlated with baseline DHEAS and androstenedione levels at the time of premature pubarche. The pattern of ovarian hyperresponsiveness was more pronounced during mid- and late puberty and was associated with hyperinsulinism [20]. Furthermore, they also observed adrenal hyperresponsiveness following ACTH stimulation before and after menarche [23]. In contrast, both ACTH and GnRH stimulation tests in a smaller sample of American girls with precocious pubarche revealed exclusively adrenal hyperresponsiveness, not supporting the hypothesis that premature pubarche was associated with prepubertal evidence of ovarian hyperandrogenism [24]. Adrenal androgen excess has been described to occur in up to 50% of patients with PCOS [25–27]. However, a more careful cluster analysis of 213 women with PCOS and 182 age-matched healthy eumenorrheic nonhirsute women indicated that the prevalence of supranormal DHEAS levels was 33.3 and 19.9% among black and white women with PCOS, respectively [28].

Two studies on precocious pubarche in girls have measured AMH, a marker of ovarian granulosa cell function that is increased in women with PCOS. In a cross-sectional study in Scottish girls with exaggerated adrenarche, AMH levels were elevated, suggesting advanced ovarian follicular development [29]. Another case-control study found normal AMH levels in Finnish prepubertal girls with PA, in contrast to prepubertal daughters of women with PCOS [30]. Intravenous GnRH tests induce lower serum AMH levels, which have a negative correlation with the increase in gonadotrophins; nevertheless, gonadotropin concentrations did not differ in our girls at early pubertal stage [31]. The lower serum concentration of AMH at the beginning of puberty in HD girls might be related to the milder hyperandrogenic milieu observed. It is known that androgen exposure results in reduced AMH, *amhr2*, and Bmp15 expression in pre-antral follicles in vitro [32].

In this study, 96 of the girls were born within the lower third of the birth weight distribution of this sample: 2.5–3 kg, and 36% of them belonged to the HD group. All our results persisted after adjusting for birth weight and BMI-SDS around 7 years of age. Furthermore, it seems that by early adulthood, the subjects born small for gestational age do not show any higher DHEAS secretion than those born appropriate for gestational age [33–35]. Four birth cohort studies have not found a relationship between low birth weight and PCOS in adult women; actually, a high birth weight was associated with PCOS [36–39]. Nevertheless, premature subjects born small for gestational age may behave differently, as they have been reported to maintain higher serum DHEAS concentrations than full-term controls until 20 years of age [40, 41].

The similar ovarian volume and prevalence of PCOM 1 year after menarche in HD and ND adolescents, who showed differences in androstenedione levels and FAI, could be attributed to the fact that PCOM is an inconsistent finding in healthy girls, especially during early puberty [42, 43].

We and others have described that the condition of PA is associated with increased BMI. An early childhood acceleration in BMI has been recently reported to be highly predictive of persistent obesity into young adulthood [44]. Therefore, lifestyle interventions including increasing physical activity and eating a healthy diet, which reduce overweight, are the cornerstone for the prevention of this condition.

Progress in defining the mechanisms that regulate adrenal androgen production has been hampered by the fact that research has focused on DHEAS, which is abundant only in some primates. As we previously mentioned, HD is not always linked to clinical manifestations of androgen actions. In fact, in the above-mentioned study, 16.6% of girls had high DHEAS, but only 50% of them had clinical manifestations [19]. Clinical manifestations may depend on hair follicle sensitivity to androgens, androgen metabolism, and other more potent adrenal androgens, which have recently been described as part of the adrenarche repertoire and may increase in a different proportion to DHEAS [45, 46].

Our study is not exempt from limitations: (1) we could have misclassified age at sexual appearance because we used the midpoint between 2 consecutive visits; however, to overcome this problem, we carried out sensitivity analysis modifying the cutoff point in the interval of the 2 visits, and we confirmed our findings; (2) in 36 girls, DHEAS determination around 7 years of age occurred at the moment of thelarche, increasing the possibility of reverse causality, but our analysis remained significant after excluding these girls; (3) the results are only applicable to girls born at term and within the birth weight range of the participants included; and (4) results of ovarian ultrasound have the inherent limitations of ultrasound performed using the transabdominal route and after 1 year of menarche. The anatomic appearance of the ovary changes with age; the volume increases during puberty and reaches the adult volume in the years following menarche [47]. Nevertheless, this study has also several strengths: (1) a unique longitudinal follow-up of unselected girls beginning at age 3–4 years; (2) visits every 6 months to assess sexual maturation; (3) a highly trained evaluator to assess sexual maturation data ($\kappa > 0.8$); and (4) methods used to assess androgens are highly specific and sensitive.

In summary, in a longitudinal sample of Chilean girls, high DHEAS levels at 7 years in normal girls without clinical symptoms or signs was associated with earlier breast and pubic hair development and menarche in addition to a mild increase in androgen levels, although within normal concentrations, throughout puberty. We believe our findings support continuous follow-up of this cohort as a unique opportunity to prospectively address the interrelationships between childhood DHEAS levels, early growth, and adiposity as determinants of ovarian function.

Acknowledgment

We thank the GOCS families and children for their participation. Everyone who contributed significantly to the work has been listed.

Statement of Ethics

The Ethics Review Board of the Institute of Nutrition and Food Technology (INTA) of the University of Chile approved the study protocol. All parents or guardians of the children provided written informed consent, and the children gave their assent.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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