ISSN 2472-1972

High DHEAS Is Associated With Earlier Pubertal Events in Girls But Not in Boys

Ana Pereira,[1] German Iñiguez,[2] Camila Corvalan,[1] and Verónica Mericq[2]

[1]Institute of Nutrition and Food Technology, University of Chile, Santiago, Chile 8360168; and [2]Institute of Maternal and Child Research, University of Chile, Santiago, Chile 7830490

Context: Premature adrenarche (PA) has been associated with increased metabolic risk.

Objective: To describe the risk of precocious thelarche (PT; <8 years), pubarche (PP; girls <8 years, boys <9 years), and gonadarche (PG; <9 years) in children with high dehydroepiandrosterone sulphate (DHEAS [HD]) vs those with normal DHEAS (ND).

Setting and Intervention: Longitudinal Chilean cohort (n = 1052, 49.9% girls). Annual clinical examination including secondary sex characteristics by Tanner staging. Logistic regression models were adjusted by age and BMI.

Main Outcome: Assess the relationship between DHEAS and premature thelarche, gonadarche, and pubarche in both sexes.

Results: At age of DHEAS determination, overweight/obesity was present in 44.3% of boys and 42.9% of girls. Incidences of any precocious event were observed in 17.2% of boys and in 25.4% of girls, presented as 8.7% of PG and 8.5% of PP in boys and as 21.3% of PT and 4.1% of PP in girls. In crude and adjusted models in boys, HD did not increase the risk of earlier pubertal events. Conversely, girls with HD had a 2.6 times greater risk of early thelarche and a three times greater risk of early pubarche compared with girls with ND concentrations.

Conclusion: In Chilean adolescents, precocious events of pubertal development were in line with the worldwide secular trend of earlier sexual maturation. HD was only associated with PT and PP in girls. Continuous follow-up of this cohort is a unique opportunity to prospectively address and analyze the interrelationships among HD, early growth, and adiposity as determinants of gonadarche, pubertal rate/sequence progression, and ovarian function.

Freeform/Key Words: adrenarche, early puberty events

Adrenarche is a gradual maturation process of the adrenal zona reticularis, the inner most zone of the adrenal cortex, beginning during the first 5 years of life [1, 2], which results in increased secretion of the adrenal androgen precursor dehydroepiandrosterone (DHEA) and its sulfate ester dehydroepiandrosterone sulphate (DHEAS). Adrenarche is clinically evident ~2 years before the onset of puberty. The initiation of adrenarche is independent of gonadal axis maturation [3], and the physiological control of this event, as well as the significance for human prepubertal development, remains unknown.

Abbreviations: BMI, body mass index; CI, confidence interval; DHEAS, dehydroepiandrosterone sulphate; HD, High DHEAS; HOMA-IR, homeostasis model assessment-estimated insulin resistance; HPG, hypothalamic-pituitary-gonadal; ND, normal DHEAS; OR, odds ratio; PA, premature adrenarche; PG, precocious gonadarche; PP, precocious pubarche; PT, precocious thelarche; SD, standard deviation; SDS, SD score.

Received 28 February 2017
Accepted 16 May 2017
First Published Online 19 May 2017

Premature adrenarche (PA), initially described in 1952 [4], is defined biochemically as an increase in adrenal androgen (DHEA and DHEAS) above the age and sex specific normal reference range, before the age of 8 years old in girls and 9 years in boys. Traditionally, elevated levels are indicated by a DHEAS level above 1086 nmol/L (above average for 6 to 8 years), although within normal limits for early puberty, and minimal if any elevation of other androgens [5]. This process is clinically recognized by the presence of signs of androgen action, including adult-type body odor, oily skin and axillary and pubic hair growth. The prevalence of PA has been reported in only a few studies, and it seems to vary among different ethnic populations [6]. Most reports have focused on pubarche, onset of pubic sexual hair growth, although other signs of androgen action are more common [5]. In a recent Finish population sample of prepubertal children aged younger than 9 years, the serum DHEAS concentrations did not differ between the sexes, and levels for biochemical adrenarche were observed in 16.6% of girls and 18.4% of boys; however, the prevalence of any clinical sign of androgen action was higher in girls than in boys (26.1% vs 10.0%; $P < 0.001$). Similarly, in a Chilean longitudinal study of obesity, we also observed no differences in DHEAS concentration by sex at age ~6.8 years [7].

Early infancy weight gain has also been associated with increased metabolic risk [8], earlier puberty [9, 10], and high DHEAS (HD) [6]. We described that obese children had twice the risk of HD and that IGF-I concentrations and not leptin were also associated with HD, independent of obesity [7].

A dimorphic effect of obesity in adrenal and gonadal function has been postulated. Obese prepubertal children (boys and girls) and only obese pubertal girls have increased testosterone and DHEAS levels, whereas androgen concentrations are not different between obese and normal weight pubertal boys [11]. In a study of 1066 children (52% obese), a significant interaction between sex and obesity for prediction of pubertal development was observed, but in the opposite direction [12]. Nevertheless, this latter observation has not been confirmed in other studies where obesity has been related to earlier pubertal events in both sexes [13, 14].

Therefore, our aim was to assess the relative contributions of DHEAS concentrations at 7 years and premature thelarche, gonadarche, and pubarche in both sexes. For this purpose, we took advantage of a previously described cohort of children enrolled in the Growth and Obesity Chilean Cohort Study, a longitudinal study of Chilean children with birth weight between 2500 and 4500 g.

1. Subjects and Methods

We performed a longitudinal study within the Growth and Obesity Chilean Cohort Study with the primary aim of assessing the association between early growth and development of adiposity and metabolic risk [15]. Briefly, the inclusion criteria were as follows: all singleton children between 3.0 and 4.9 years attending Chilean National Nursery School Council Program from the south east area of Santiago, Chile in 2006; gestational age 37 to 42 weeks; birth weight ≥2500 g and ≤4500 g (data retrieved from medical registries); and with no physical or psychological conditions that could severely affect growth. Eighty-five percent of the children agreed to participate (n = 1190). We did not observe substantial differences in age, sex, birth, and anthropometry at enrollment between participants and nonparticipants. Thereafter, annual evaluations have been conducted (anthropometry, body composition, skeletal and hormonal maturation, and metabolic/inflammatory markers) and, since 2009, twice yearly visits were initiated to assess secondary sex characteristics. Maternal age at menarche was self-reported by the mother.

In 2009, 1044 children of the original cohort were evaluated (~88%). For the current analyses, we excluded a total of 58 children, 36 for whom no blood sample was obtained due to difficulties and 22 children without sexual maturation data. Thus, our final sample size was 986 children. The study protocol was approved by the Institutional Review Board of the Institute of Nutrition and Food Technology of the University of Chile. Written informed consent was obtained from all parents or guardians of the children and assent from the children.

A. Clinical Adrenal and Pubertal Development

At age ~7 years, a single pediatric endocrinologist (VM) assessed breast and genital development by palpation and classified breast and testes according to Tanner stages [16, 17]. The same endocrinologist evaluated the presence of clinical signs of androgen action. Thereafter, every 6 months, secondary sex characteristics were evaluated by a single dietitian (same sex) trained specially for this purpose, with permanent supervision of a single pediatric endocrinologist (VM). In girls, breasts were evaluated by inspection and palpation according to the Tanner scale [16] and, in boys, genitalia were evaluated by palpation using the Prader orchidometer [17]. In both sexes, pubarche was assessed based on the Tanner scale. Concordance between the dietitian and pediatric endocrinologist was 0.9 for breast [18] and genitalia evaluation.

Age at thelarche, gonadarche, and pubarche onset was defined as the midpoint of two consecutive visits, the last visit without signs of sexual development (breast, genital, or pubic hair) and the first visit in which one of these signs were detected. Thus, we defined precocious thelarche (PT) when this event (midpoint between visits) appeared before the age of 8 years, precocious gonadarche (PG) as a testicular volume >3 mL at age before 9 years, and precocious pubarche (PP) when pubic hair appeared before the age of 8 years in girls or before 9 years in boys.

B. Anthropometric Measures

Weight and height were collected using standardized protocols (barefoot and light clothes) by two dietitians (one female, one male) with inter- and intrarater correlation coefficients over 0.80 for all measurements. Weight was measured with a portable electronic scale (Seca 770), with precision of 0.1 kg, and height was measured with a portable stadiometer (Harpenden 603) to the nearest 0.1 cm. A complete description of anthropometry methodology is found elsewhere [7].

C. Blood Sample at 7 Years

A trained nurse collected a fasting venous sample (8 to 12 hours) from the children upon arrival to the Institute of Nutrition and Food Technology outpatient clinic. Mothers were contacted the day before sample drawing to confirm the absence of fever (>37.5°C) or symptoms of acute infection in the children. Serum glucose concentrations were assessed by enzymatic colorimetric techniques (HUMAN, Gesellschaft fur Biochemica und Diagnostica, Wiesbaden, Germany) and serum insulin, using a radioimmunoassay kit (Linco Research, Inc., St. Charles, MO). Serum leptin and adiponectin were measured by commercial radioimmunoassay (Millipore, Merck, Darmstadt, Germany). Analyses were conducted at the Nutrition Laboratory of the Catholic University of Chile. This laboratory conducts daily assessments of the accuracy of the measurements by using UNITY quality control software (Bio-Rad Laboratories, Inc., Hercules, CA). DHEAS and IGF-I analyses were conducted at the Institute of Maternal and Child Research University of Chile. Serum DHEA-S was determined by competitive specific binding RIA supplied by Diagnostic System Laboratories (Webster, TX); intra- and interassay coefficient of variation (CVs) were 3.5% and 5.1%, respectively. Serum IGF-I was measured by using a standardized locally developed radioimmunoassay requiring sample extraction as a first step (sensitivity: 5 ng/mL; intra- and interassay CVs: 8.6% and 10.2%, respectively) [19].

D. Computed Indices

Body mass index (BMI) was estimated by dividing weight (kg) by height squared (m^2). We estimated height-for-age and BMI-for-age based on the World Health Organization 2006 standards and the World Health Organization 2007 growth reference [20]. We defined obesity as BMI standard deviation score (SDS) ≥ 2. Biochemical indicator of HD defined as DHEAS

concentration drawn at age 7 based on sample distribution (75th percentile); cutoffs were 42.0 μg/dL for girls and 45.1 μg/dL for boys [7]. The homeostasis model assessment-estimated insulin resistance (HOMA-IR) was calculated as fasting glucose (mmol/L) X fasting insulin (mU/mL)/22.5.

E. Statistical Analysis

Descriptive analysis (mean, SD, percentage) of anthropometric, hormonal characteristics, and sexual maturation data were performed by stratifying PA. Statistical differences among groups were assessed using χ^2 and Student t test accordingly and were considered significant at a P value < 0.05.

We performed logistic regression models [odds ratio (OR) and 95% confidence interval (CI)] to assess the association between precocious adrenarche and PG, PT, and PP (separately by sex). The OR was adjusted in three different models. Model 1 was adjusted only by age at DHEAS sampling and birth weight. Model 2 was adjusted by birth weight, age, and BMI SDS at DHEAS sampling. Model 3 was adjusted by birth weight, age, BMI SDS, insulin, IGF-1, and leptin at DHEAS sampling.

As age at thelarche, gonadarche, and pubarche onset was defined as the midpoint of two consecutive visits, it could occur within that period of time. To assess this error, we performed a sensitivity analyses, moving the age of pubertal onset to the beginning and to the end of this period.

2. Results

We collected blood samples from 494 boys and 492 girls at 7 years old and characterized the participants as HD or normal DHEAS (ND). HD was present in 26.1% of the samples and we did not observe differences across sexes. At follow-up, mean age 12 years, 99% of the girls and 60% of the boys reached a pubertal stage ≥ Tanner II. A complete description of the anthropometric and hormonal-metabolic characteristics of the study population at 2009 (age at DHEAS sampling) and percentage of PP, PT, and PG are show in Table 1.

A. Boys

Boys with HD showed no difference in the incidence of PG and PP compared with boys who had ND concentrations; however, they were more obese, overweight, and taller and had a higher IGF-I, and leptin (Table 2). Boys with PG had a higher birth weight, were taller, and had a higher BMI SDS at age ~7 compared with those who did not show earlier gonadal development (data not shown). Four out of 14 boys with PG and HD had PP (Table 2). In boys with PP, no differences in anthropometric, hormonal, or metabolic data were found at this age that could predict earlier development of pubic hair (data not shown in table).

In crude and adjusted models in boys, HD did not increase the risk of earlier pubertal events (gonadarche and pubarche), even after adjusting for birth weight, BMI SDS, insulin, leptin, and IGF-I at age 7 (DHEAS sampling; Table 3). The results were similar after performing a sensitivity analysis, redefining age at puberty onset as the upper or lower limit of the interval of the two consecutive visits.

B. Girls

In contrast, girls with HD had an approximately two times greater risk of early thelarche and approximately three times greater risk of early pubarche, compared with girls with ND concentrations. Similar to boys, girls were more obese and overweight and had a higher IGF-I concentrations at age 7 (Table 2).

Four out of the 45 girls who had PT and HD had PP (Table 2). Girls with PP were taller and had higher BMI SDS than those who did not present earlier development of pubic hair (data not shown). In contrast to boys, HD increased the risk of early thelarche significantly in both

Table 1. Anthropometric and Hormonal-Metabolic Description of Study Population at 2009 and Percentage of PP, PT, and PG

	Boys, n = 494	Girls, n = 492
	Mean (SD)	Mean (SD)
Age at 2009, y	6.8 (0.4)	6.8 (0.4)
Birth weight, kg	3.4 (0.4)	3.4 (0.4)
Birth weight *z* score	0.08 (1.9)	0.18 (1.06)
Height, cm	121.2 (5.5)	120.4 (5.4)
Height *z* score	0.1 (0.9)	0.2 (0.9)
Weight, kg	25.4 (4.9)	25.1 (4.7)
BMI *z* score	0.9 (1.3)	0.9 (1.1)
Obese (>2 SD), n (%)	106 (21.5%)	70 (14.2%)
Overweight (>1 SD), n (%)	219 (44.3%)	211 (42.9%)
Insulin, μIU/mL	5.4 (1.3)	5.6 (1.7)
HOMA	1.2 (0.3)	1.2 (0.4)
IGF-I SDS	−0.1 (0.7)	−0.5 (0.7)
IGF-I, nmol/L	24.6 (8.16)	23.1 (5.7)
Leptin, nmol/L	0.36 (0.24)	0.38 (0.26)
Adiponectin, μg/mL	18.4 (7.2)	17.5 (6.1)
Precocious Adrenarche, n (%)	126 (25.5%)	131 (26.6%)
PG, n (%)	43 (8.7%)	—
PT, n (%)		105 (21.3%)
PP, n (%)	42 (8.5%)	20 (4.1%)
PG and PP, n (%)	10 (2.0%)	—
PT and PP, n (%)		8 (1.6%)

crude and adjusted models. Thirty-six girls had Tanner II breast at the moment of DHEAS determination [OR 1.83 crude model (1.07 to 3.07), model 1 OR 1.91 (1.08 to 3.30), model 2 OR 1.97 (1.11 to 3.45), and model 3 OR 1.87 (1.04 to 3.32)]. HD also determined earlier pubarche in girls, but the association was significant only in the crude and adjusted model by age and birth weight. However, the magnitude of the association remained similar after further adjustment (the results were similar after the sensitivity analysis).

3. Discussion

In this longitudinal population-based study, higher levels of DHEAS at age ~6.8 years were associated with a higher BMI in both boys and girls, but only in girls with an earlier appearance of secondary sex characteristics. This finding remained true even after adjusting for IGF-1 and other relevant metabolic hormones, suggesting that these adrenal hormones directly promote earlier thelarche. In addition to increased DHEAS, both girls and boys had increased IGF-I, and in boys a taller stature and a greater concentration of leptin was observed.

This sex dimorphism in the association between HD and earlier appearance of sexual characteristics points toward the peripheral conversion of adrenal hormones through aromatase conversion to estrogen precursors, which play a role in increasing gonadotropin-releasing hormone secretion, follicle-stimulating hormone, and luteinizing hormone, finally activating the ovary [21]. The timing of puberty is precisely controlled by a plethora of endogenous signals and environmental factors that impinge at different levels of the hypothalamic-pituitary-gonadal (HPG or reproductive) axis. Accordingly, puberty has been regarded not only as a fundamental developmental event but also as a sensor of the proper interplay between genes and the environment along development. Additionally, pubertal timing is highly polygenic and a number of identified loci underlie both pubertal timing and related traits such as height and BMI [22]. Studies undertaken in many species indicate that

Table 2. Description of PG, PT, PP and Anthropometric and Hormonal Characteristics Stratified by Sex and With or Without Precocious Adrenarche

	Boys			Girls		
	HD, n = 126	ND, n = 368		HD, n = 131	ND, n = 361	
	Mean (SD)	Mean (SD)	P Value	Mean (SD)	Mean (SD)	P Value
PG, n (%)	14 (11.1)	29 (7.9)	0.3			
PT, n (%)				45 (34.4)	60 (16.6)	<0.001
PP, n (%)	12 (9.5)	30 (8.2)	0.6	10 (7.6)	10 (2.8)	0.02
PG and PP, n (%)	4 (3.2)	6 (1.6)	0.28[a]			
PT and PP, n (%)				4 (3.1)	4 (1.1)	0.07[a]
BMI, SDS	1.4(1.31)	0.79 (1.22)	<0.001	1.16 (1.09)	0.75 (1.03)	0.0002
Obesity, n (%)	42 (33.3)	64 (17.4)	<0.001	26 (19.8)	44 (12.2)	0.03
Overweight, n (%)	71 (56.3)	148 (40.2)	<0.001	69 (52.7)	142 (39.3)	<0.001
Birth weight, kg	3.39 (0.44)	3.45 (0.41)	0.1	3.31 (0.38)	3.39 (0.39)	0.05
Birth weight *z* score	−0.01 (1.14)	0.11 (1.08)	0.31	0.09 (1.04)	0.21 (1.06)	0.26
Height, SDS	0.35 (0.85)	0.06 (0.95)	0.001	0.3 (0.99)	0.15 (0.86)	0.11
Insulin, pM/L	39.8 (10.1)	37.2 (8.3)	0.2	39.0 (9.9)	38.8 (12.2)	0.81
HOMA-IR	1.25 (0.37)	1.19 (0.3)	0.1	1.24 (0.36)	1.23 (0.44)	0.76
IGF-I, nmol/L	26.20 (8.45)	24.04 (7.9)	0.01	24.4 (6.45)	22.55 (5.34)	0.002
IGF-I, SDS	0.07 (0.75)	−0.20 (0.66)	0.008	−0.32 (0.77)	−0.54 (0.69)	0.004
Leptin, nmol/L	0.4025 (0.26)	0.34 (0.23)	0.02	0.39 (0.26)	0.37 (0.26)	0.58
Adiponectin, ng/mL	17.91 (6.85)	18.52 (7.33)	0.4	17.52 (6.03)	17.48 (6.18)	0.96

[a]Fisher test.

kisspeptin-Gpr54 signaling is essential for the activation of gonadotropin-releasing hormone neurons to initiate puberty. This has recently been shown to be dependent upon circulating estradiol concentrations [21], supporting our hypothesis that increasing peripheral estrogens could sensitize the HPG axis.

Conversely, the earlier appearance of secondary sex characteristics might be dissociated from HPG maturation and may only reflect the peripheral action of DHEAS and estrogen metabolites. Recent data in a longitudinal study of 252 peripubertal girls in the United States show that DHEAS concentrations increased 24 months before breast development and estrone between 12 to 18 months before breast development; whereas estradiol and testosterone increased (mostly from ovarian source) more closely to the time of breast development [23]. Another interesting fact is that children with HD have a higher percent of obesity and overweight, and in this aforementioned study, girls with a greater BMI had a lower estradiol concentrations at onset of breast development, as well as 6 months after thelarche, pointing toward a peripheral source of sex steroids more than to a centrally active HPG as a cause of thelarche [23]. In a recent paper on the effect of obesity in puberty onset [12], there was a lack of association between adiposity and ovarian volume in girls, suggesting that the advanced breast development observed among early pubertal obese girls may be primarily due to peripheral conversion of relatively inactive androgens to more bioactive estrogens by aromatase in adipose tissues. We recently published that higher levels of estradiol equivalents in prepubertal girls were associated with an earlier thelarche, which remained significant after adjusting for BMI or other adiposity markers, suggesting that these associations were not mediated through adiposity [24]. These observations are also supported by evidence suggesting that age at the gonadotropin and sex steroid surges have not changed in population studies where age at thelarche has declined [25]. It may also be possible that some of our PT girls have HPG activation, whereas, in others, thelarche is mainly peripheral. In our sample of girls with HD, 3.1% presented with PT and PP together, suggesting HPG activation, at least in this population. Data on menarche will help to elucidate whether the earlier appearance of sex characteristics is a reflection of HPG activation. Nonetheless, evidence from the Avon

Table 3. Crude and Adjusted OR and 95% CI Between HD and Precocious Adrenarche and PG, PT, and PP

	OR	95% CI	OR 1[a]	95% CI	OR 2[b]	95% CI	OR 3[c]	95% CI
Male PG	1.46	0.73–2.81	1.58	0.76–3.16	1.23	0.57–2.53	1.13	0.37–3.19
Male PP	1.18	0.57;2.34	1.39	0.63–2.93	1.22	0.54–2.6	1.5	0.46–4.53
Female PT	2.63	1.66;4.13	2.67	1.65–4.31	2.48	1.52–4.04	2.37	1.44–3.90
Female PP	2.9	1.16;7.23	2.87	1.08–7.53	2.58	0.94–6.98	2.47	0.90–6.69

[a]Adjusted by age and birth weight.
[b]Adjusted by model 1 + BMI SDS.
[c]Adjusted by model 2 + insulin, leptin, and IGF-1.

Longitudinal Study of Parents and Children study of 329 girls showed that higher DHEAS at age 8 predicted earlier menarche [26], which is more in line with a centrally activated HPG axis.

Leptin is an essential signal in the control of body weight homeostasis, and its levels in circulation are proportional to the size of fat stores. Our results showing that HD is associated with earlier pubertal events in girls were, nonetheless, adjusted for leptin and insulin and are in agreement with pharmacological studies in humans and rodents with leptin deficiency, documenting that, although leptin is indispensable for puberty to proceed, leptin alone cannot trigger early puberty [27].

Another interesting finding of the current study is the increase in the percent of earlier thelarche (21.3%). In a 2004 cross-sectional analysis performed in our country with 758 girls aged 5.8 to 16.1 years, 16% of girls had thelarche [28] at ages below 8 years old. Our data are concordant with recent Danish longitudinal data describing a recent decline in age at thelarche in a decade [25].

Less data on pubertal maturation are available in boys, in part because pubertal development in boys is more difficult to ascertain on a large scale. In the current study, an increased BMI, mainly overweight, but not HD, was the only factor associated with earlier gonadal maturation. Interestingly, at high concentrations, as expected in obesity, leptin can directly inhibit gonadal function [29, 30]. Although available, data are mixed, a U-shaped relationship could help understand the controversy on the effect of BMI in male puberty [12, 31–33]. Although this was initially considered counterintuitive, the inhibitory actions of leptin may explain part of the hypogonadal state frequently observed in morbidly obese patients [12].

Pubic hair development is mediated by androgen exposure modulated by androgen receptor response. Among girls, HD more than doubled the risk of PP. Overall, in our sample the frequency of PP was 4.1% in girls and 8.5% in boys, which is higher than in previous cross-sectional Chilean studies in girls [28] and boys [34]. Pubic hair is highly dependent on ethnicity [35], and these differences appear to be at least in part mediated by the androgen receptor gene methylation pattern, as well as the presence of shorter CAG trinucleotide repeat [36]. Interestingly, in boys HD did not increase the risk of PP. DHEAS is a weak androgen and together with a lower sensitivity to androgens may not be enough to promote sexual hair growth in boys. Indeed, in a clinical evaluation of body hair in our country, the authors found that women in our country were more likely to be hairless than European or North American women and proposed a lower Ferriman and Gallwey score cutoff for hirsutism diagnosis [37].On the other hand, we cannot exclude that girls with PP alone were starting HPG axis maturation through this pathway and not through thelarche.

Our study is not exempt from limitations: (1) lack of assessment of luteinizing hormone and follicle-stimulating hormone at 2009, at age when DHEAS were obtained; (2) we could have misclassified age at sexual appearance because we use the midpoint of two consecutive visits; to overcome this problem, we carried out sensitivity analysis modifying the cutoff point in the interval of the two visits; and (3) in 36 girls, DHEAS determination occurred at the moment of thelarche, raising the possibility of reverse causality. Nevertheless, the association remained significant after excluding these girls. The study had the following strengths as well: (1)

longitudinal follow-up since age 3 to 4 years; (2) visits every 6 months to assess sexual maturation; and (3) a highly trained evaluator to assess sexual maturation data ($\kappa > 0.8$).

4. Conclusions

In Chilean adolescents, precocious events of pubertal development were in line with worldwide secular trend of earlier sexual maturation. HD was only associated with PT and PP in girls. Continuous follow-up of this cohort is a unique opportunity to prospectively address the interrelationships among PA, early growth, adiposity as determinants of gonadarche, pubertal rate, and sequence progression and ovarian function.

Acknowledgments

We thank all the study personnel, particularly Daniela Gonzalez, for field work coordination.

Address all correspondence to: Verónica Mericq, MD, School of Medicine, University of Chile, Maternal and Child Research Institute, Santiago de Chile, PO Box 226–3, Santiago, Chile. E-mail: vmericq@med.uchile.cl.

This work was supported by FONDECYT Grants 1140447 and 1120326.

Disclosure Summary: The authors have nothing to disclose.

References and Notes

1. Palmert MR, Hayden DL, Mansfield MJ, Crigler JF, Jr, Crowley WF, Jr, Chandler DW, Boepple PA. The longitudinal study of adrenal maturation during gonadal suppression: evidence that adrenarche is a gradual process. *J Clin Endocrinol Metab*. 2001;**86**(9):4536–4542.
2. Remer T, Boye KR, Hartmann MF, Wudy SA. Urinary markers of adrenarche: reference values in healthy subjects, aged 3-18 years. *J Clin Endocrinol Metab*. 2005;**90**(4):2015–2021.
3. Sklar CA, Kaplan SL, Grumbach MM. Evidence for dissociation between adrenarche and gonadarche: studies in patients with idiopathic precocious puberty, gonadal dysgenesis, isolated gonadotropin deficiency, and constitutionally delayed growth and adolescence. *J Clin Endocrinol Metab*. 1980;**51**(3):548–556.
4. Silverman SH, Migeon C, Rosemberg E, Wilkins L. Precocious growth of sexual hair without other secondary sexual development; premature pubarche, a constitutional variation of adolescence. *Pediatrics*. 1952;**10**(4):426–432.
5. Utriainen P, Jääskeläinen J, Romppanen J, Voutilainen R. Childhood metabolic syndrome and its components in premature adrenarche. *J Clin Endocrinol Metab*. 2007;**92**(11):4282–4285.
6. Ong KK, Potau N, Petry CJ, Jones R, Ness AR, Honour JW, de Zegher F, Ibáñez L, Dunger DB; Avon Longitudinal Study of Parents and Children Study Team. Opposing influences of prenatal and postnatal weight gain on adrenarche in normal boys and girls. *J Clin Endocrinol Metab*. 2004;**89**(6):2647–2651.
7. Corvalán C, Uauy R, Mericq V. Obesity is positively associated with dehydroepiandrosterone sulfate concentrations at 7 y in Chilean children of normal birth weight. *Am J Clin Nutr*. 2013;**97**(2):318–325.
8. Kain J, Corvalán C, Lera L, Galván M, Uauy R. Accelerated growth in early life and obesity in preschool Chilean children. *Obesity (Silver Spring)*. 2009;**17**(8):1603–1608.
9. Wang Y, Dinse GE, Rogan WJ. Birth weight, early weight gain and pubertal maturation: a longitudinal study. *Pediatr Obes*. 2012;**7**(2):101–109.
10. Ong KK, Emmett P, Northstone K, Golding J, Rogers I, Ness AR, Wells JC, Dunger DB. Infancy weight gain predicts childhood body fat and age at menarche in girls. *J Clin Endocrinol Metab*. 2009;**94**(5):1527–1532.
11. Reinehr T, de Sousa G, Roth CL, Andler W. Androgens before and after weight loss in obese children. *J Clin Endocrinol Metab*. 2005;**90**(10):5588–5595.
12. Crocker MK, Stern EA, Sedaka NM, Shomaker LB, Brady SM, Ali AH, Shawker TH, Hubbard VS, Yanovski JA. Sexual dimorphisms in the associations of BMI and body fat with indices of pubertal development in girls and boys. *J Clin Endocrinol Metab*. 2014;**99**(8):E1519–E1529.

13. Mouritsen A, Aksglaede L, Soerensen K, Hagen CP, Petersen JH, Main KM, Juul A. The pubertal transition in 179 healthy Danish children: associations between pubarche, adrenarche, gonadarche, and body composition. *Eur J Endocrinol*. 2012;**168**(2):129–136.
14. Aksglaede L, Juul A, Olsen LW, Sørensen TI. Age at puberty and the emerging obesity epidemic. *PLoS One*. 2009;**4**(12):e8450.
15. Corvalán C, Uauy R, Stein AD, Kain J, Martorell R. Effect of growth on cardiometabolic status at 4 y of age. *Am J Clin Nutr*. 2009;**90**(3):547–555.
16. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Arch Dis Child*. 1969; **44**(235):291–303.
17. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Arch Dis Child*. 1970; **45**(239):13–23.
18. Pereira A, Garmendia ML, González D, Kain J, Mericq V, Uauy R, Corvalán C. Breast bud detection: a validation study in the Chilean growth obesity cohort study. *BMC Womens Health*. 2014;**14**:96.
19. Iñiguez G, Villavicencio A, Gabler F, Palomino A, Vega M. Effect of nitric oxide on the expression of insulin-like growth factors and the insulin-like growth factor binding proteins throughout the lifespan of the human corpus luteum. *Reproduction*. 2001;**122**(6):865–873.
20. de Onis M, Garza C, Victora CG, Onyango AW, Frongillo EA, Martines J. The WHO Multicentre Growth Reference Study: planning, study design, and methodology. *Food Nutr Bull*. 2004; **25**(1, Suppl)S15–S26.
21. Clarkson J, Boon WC, Simpson ER, Herbison AE. Postnatal development of an estradiol-kisspeptin positive feedback mechanism implicated in puberty onset. *Endocrinology*. 2009;**150**(7):3214–3220.
22. Day FR, Helgason H, Chasman DI, Rose LM, Loh PR, Scott RA, Helgason A, Kong A, Masson G, Magnusson OT, Gudbjartsson D, Thorsteinsdottir U, Buring JE, Ridker PM, Sulem P, Stefansson K, Ong KK, Perry JR. Physical and neurobehavioral determinants of reproductive onset and success. *Nat Genet*. 2016;**48**(6):617–623.
23. Biro FM, Pinney SM, Huang B, Baker ER, Walt Chandler D, Dorn LD. Hormone changes in peripubertal girls. *J Clin Endocrinol Metab*. 2014;**99**(10):3829–3835.
24. Pereira A, Corvalán C, Uauy R, Klein KO, Mericq V. Ultrasensitive estrogen levels at 7 years of age predict earlier thelarche: evidence from girls of the growth and obesity Chilean cohort. *Eur J Endocrinol*. 2015;**173**(6):835–842.
25. Aksglaede L, Sørensen K, Petersen JH, Skakkebaek NE, Juul A. Recent decline in age at breast development: the Copenhagen Puberty Study. *Pediatrics*. 2009;**123**(5):e932–e939.
26. Thankamony A, Ong KK, Ahmed ML, Ness AR, Holly JM, Dunger DB. Higher levels of IGF-I and adrenal androgens at age 8 years are associated with earlier age at menarche in girls. *J Clin Endocrinol Metab*. 2012;**97**(5):E786–E790.
27. Roa J, García-Galiano D, Castellano JM, Gaytan F, Pinilla L, Tena-Sempere M. Metabolic control of puberty onset: new players, new mechanisms. *Mol Cell Endocrinol*. 2010;**324**(1-2):87–94.
28. Codner E, Unanue N, Gaete X, Barrera A, Mook-Kanamori D, Bazaes R, Avila A, Cassorla F. [Age of pubertal events in Chilean school age girls and its relationship with socioeconomic status and body mass index]. *Rev Med Chil*. 2004;**132**(7):801–808.
29. Caprio M, Fabbrini E, Isidori AM, Aversa A, Fabbri A. Leptin in reproduction. *Trends Endocrinol Metab*. 2001;**12**(2):65–72.
30. Tena-Sempere M, Pinilla L, González LC, Diéguez C, Casanueva FF, Aguilar E. Leptin inhibits testosterone secretion from adult rat testis in vitro. *J Endocrinol*. 1999;**161**(2):211–218.
31. De Leonibus C, Marcovecchio ML, Chiavaroli V, de Giorgis T, Chiarelli F, Mohn A. Timing of puberty and physical growth in obese children: a longitudinal study in boys and girls. *Pediatr Obes*. 2014;**9**(4):292–299.
32. Lee JM, Wasserman R, Kaciroti N, Gebremariam A, Steffes J, Dowshen S, Harris D, Serwint J, Abney D, Smitherman L, Reiter E, Herman-Giddens ME. Timing of Puberty in Overweight Versus Obese Boys. *Pediatrics*. 2016;**137**(2):e20150164.
33. Sørensen K, Aksglaede L, Petersen JH, Juul A. Recent changes in pubertal timing in healthy Danish boys: associations with body mass index. *J Clin Endocrinol Metab*. 2010;**95**(1):263–270.
34. Gaete X, García R, Riquelme J, Codner E. [Age of onset of puberty in Chilean boys according to testicular volume and Tanner stage]. *Rev Med Chil*. 2015;**143**(3):297–303.
35. Rosenfield RL, Lipton RB, Drum ML. Thelarche, pubarche, and menarche attainment in children with normal and elevated body mass index. *Pediatrics*. 2009;**123**(1):84–88.
36. Vottero A, Capelletti M, Giuliodori S, Viani I, Ziveri M, Neri TM, Bernasconi S, Ghizzoni L. Decreased androgen receptor gene methylation in premature pubarche: a novel pathogenetic mechanism? *J Clin Endocrinol Metab*. 2006;**91**(3):968–972.
37. Téllez R, Frenkel J. [Clinical evaluation of body hair in healthy women]. *Rev Med Chil*. 1995;**123**(11): 1349–1354.