Higher Levels of IGF-I and Adrenal Androgens at Age 8 Years Are Associated with Earlier Age at Menarche in Girls

Ajay Thankamony, Ken K. Ong, M. Lynn Ahmed, Andrew R. Ness, Jeff M. P. Holly, and David B. Dunger

Department of Paediatrics (A.T., K.K.O., D.B.D.), University of Cambridge, Cambridge CB2 0QQ, United Kingdom; Medical Research Council Epidemiology Unit (K.K.O.), Institute of Metabolic Science, Cambridge CB2 0QQ, United Kingdom; National Institute for Health Research (D.B.D.), Cambridge Comprehensive Biomedical Research Centre, Cambridge CB2 0QQ, United Kingdom; Department of Paediatrics (M.L.A.), University of Oxford, Oxford OX1 2JD, United Kingdom; and School of Oral and Dental Sciences (A.R.N.), and School of Clinical Science (J.M.P.H.), University of Bristol, Bristol BS8 1TZ, United Kingdom

Context: Earlier age at menarche is associated with rapid infancy weight gain and childhood obesity. The role of hormone levels in mediating these associations is unclear.

Objective: The aim of this study was to identify childhood hormone levels at age 8 yr that are associated with early menarche, independent of body size.

Design, Settings, and Subjects: A total of 329 girls from a prospective United Kingdom birth cohort study provided blood samples at mean age 8.1 yr (range, 8.0–8.5) for hormone measurements and were followed longitudinally to establish age at menarche.

Main Outcome Measures: Fasting plasma levels of IGF-I, androstenedione, dehydroepiandrosterone sulfate (DHEAS), leptin, insulin, IGF binding protein-1, and SHBG were measured. Age at menarche was reported by questionnaire and categorized as before 12.0, 12.0–13.0, or later than 13 yr.

Results: Earlier menarche was associated with greater body weight, height, and body mass index at age 8 yr (all P-trend <0.001). Before adjustment for body size, earlier menarche was associated with higher levels of IGF-I, androstenedione, DHEAS, leptin, and fasting insulin, and with lower levels of IGF binding protein-1 and SHBG at age 8 yr (all $P < 0.01$). After adjustment for body mass index and height at age 8 yr, only IGF-I ($P = 0.004$), androstenedione ($P = 0.01$), and DHEAS ($P = 0.01$) remained associated with earlier menarche.

Conclusions: Associations between higher levels of IGF-I and adrenal androgens at age 8 yr with earlier menarche, independent of body size, support functional roles of these hormones in regulating puberty timing in girls. Higher levels of these hormones reported in children who exhibited rapid weight gain during infancy may indicate their role in developmental pathways leading to earlier sexual maturation. **(*J Clin Endocrinol Metab* 97: E786–E790, 2012)**

The mechanisms of pubertal timing in humans are not well defined and are thought to be mediated through complex interactions of neural and endocrine signals resulting in stimulation of the GnRH pulse regulator. Although marked changes in pubertal onset are seen in disorders of GH, IGF-I, leptin, insulin, and adrenal androgens (1), the physiological roles of these hormones in regulating pubertal timing are uncertain. Earlier age at menarche has been associated with low birth weight, rapid weight gain during infancy, and childhood obesity, sug-

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2011-3261 Received November 30, 2011. Accepted January 30, 2012.
First Published Online March 14, 2012

Abbreviations: BMI, Body mass index; CV, coefficient of variation; DHEAS, dehydroepiandrosterone sulfate; IGFBP-1, IGF binding protein-1.

TABLE 1. Body size at age 8 yr by subsequent age at menarche in girls

	Age at menarche (yr)			
	<12	12–13	13+	*P*-trend
n	71	74	184	
Body size at 8 yr (mean)				
Weight (kg)	30.0 (27.0–35.3)	28.0 (26.0–31.3)	26.0 (24.0–30.0)	<0.00001
Height (cm)	133 (130–136)	130 (126–135)	129 (125–133)	<0.00001
BMI (kg/m^2)	17.0 (16.0–19.3)	16.6 (15.4–18.1)	16.1 (15.0–17.2)	<0.00001
Waist circumference (cm)	57 (54–63)	56 (54–61)	55 (52–58)	<0.00001

Data are expressed as median (interquartile range). *P* values represent tests of the trends across the three age-at-menarche groups and is adjusted for age.

gesting a role of developmental programming in the regulation of pubertal timing (2). However, the hormonal changes that potentially mediate these associations have not been defined. The aim of this study was to identify childhood hormonal predictors of earlier menarche that are independent of body size.

Subjects and Methods

Participants

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a Bristol (UK)-based prospective study of 14,541 newborns recruited from all pregnancies with expected dates of delivery between April 1991 and December 1992 and is described in detail elsewhere (3). Ethics approval for the study was obtained from the ALSPAC Law and Ethics Committee and the Local Research Ethics Committee. Signed consent was obtained from a parent, and verbal assent was obtained from the child.

Data and sample collection

Anthropometric measurements and an overnight fasting blood sample were collected at a research clinic at age 8 yr. Height was measured using a Harpenden stadiometer (Holtain Ltd., Dyfed, UK), body weight was measured using electronic scales, and waist circumference was measured midway between the lowest rib and the iliac crest by tape measure (Harpenden anthropometric tapes, Holtain Ltd.). Body mass index (BMI) was calculated as weight (kilograms)/height (meters)^2. At a later research clinic at mean age 12.9 yr (interquartile range, 12.8–13.0 yr), girls were asked whether they had started their menstrual periods and, if so, at what age. Some missing data on age at menarche at this clinic were imputed from data collected at ages 11 and 13 yr. Age at menarche was therefore categorized into three groups: less than 12.0, 12.0–13.0, and more than 13.0 yr; the last category included those who had not yet started menstruation. Breast development stage was self-reported on postal questionnaires sent to parents at age 8 yr (4).

Assays

IGF-I levels were determined by RIA using a monoclonal antibody (Blood Products, Elstree, UK) and recombinant peptide (Pharmacia, Stockholm, Sweden) for standard and tracer after iodination using the chloramine-T method. Samples were analyzed after acid-acetone extraction and IGF-II saturation, and the intraassay and interassay coefficients of variation (CV) were 6.7 and 12%, respectively. IGF binding protein-1 (IGFBP-1) levels were measured by ELISA (Diagnostic Systems Laboratories, Oxford, UK) with intraassay and interassay CV of 5.3 and 5.1%, respectively. Androstenedione levels were measured by RIA (Diagnostic Systems Laboratories) with intraassay and interassay CV of 6.3 and 9.3%, respectively. Dehydroepiandrosterone sulfate (DHEAS) was assayed by immunochemiluminescence (Immulite assay; Diagnostics Products Corporation, Madrid, Spain). Intraassay and interassay CV were 5.6 and 10.1%, respectively. SHBG and leptin levels were measured using ELISA (DRG Instruments GmbH, Marburg, Germany) with interassay CV of 13 and 11.5%, respectively. Glucose was measured by the glucose oxidase method on the YSI 2300 STAT Plus analyzer (YSI Inc., Farnborough, UK). At 4.1 mmol/liter, the intraassay and interassay CV were 1.5 and 2.8%, respectively. Insulin was measured by ELISA (Diagnostic Systems Laboratories); the intraassay CV were 4.4 and 5.1% at 10.3 and 35.8 mU/liter, and equivalent interassay CV were 8.7 and 2.9%, respectively.

Statistics

Differences in variables between categories of age at menarche were tested using multivariable linear regression, adjusting for exact age at the 8-yr-old baseline visit. Further models were performed, adjusting for BMI and height at 8 yr, and also excluding girls with self-reported breast development at age 8 yr. Analyses were performed using SPSS for Windows (version 15.0; SPSS Inc., Chicago, IL).

Results

This report is based on a complete case analysis of the 329 girls with data on age of menarche and hormone measurements at age 8 yr (mean, 8.1 yr; range, 8.0–8.5). Earlier age at menarche was associated with greater body weight, height, and BMI at age 8 yr (Table 1). Before adjustment for body size, earlier menarche was associated with higher levels of IGF-I, androstenedione, DHEAS, leptin, and fasting insulin, and with lower levels of IGFBP-1and SHBG at age 8 yr (all $P < 0.01$) (Table 2 and Supplemental Figure 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). However, after adjustment for BMI and height at 8 yr, only IGF-I ($P = 0.004$), androstenedione ($P = 0.01$), and DHEAS ($P = 0.01$) remained associated with earlier menarche (Table

TABLE 2. Hormone levels at age 8 yr by girl's age at menarche

	Age at menarche (yr)			Linear regression models[a]		
	<12	12–13	13+	Basic model	Adjusted for BMI and height	Adjusted for BMI, height, and prepubertal at baseline[c]
n	71	74	184			
IGF-I (ng/ml)	185 (132–214)	172 (121–210)	149 (115–174)	6.8%, $P < 0.001$	4.8%, $P = 0.004$	7.8%, $P < 0.001$
Androstenedione (ng/dl)	62.2 (42.4–86.4)	53.6 (37.4–79.7)	48.6 (33.8–64.8)	4.7%, $P < 0.001$[b]	2.2%, $P = 0.01$[b]	1.9%, $P = 0.02$[b]
DHEAS (μg/dl)	29.2 (13.6–50.9)	20.9 (9.4–35.2)	15.9 (10.2–27.1)	6.6%, $P < 0.001$[b]	1.9%, $P = 0.01$[b]	2.0%, $P = 0.02$[b]
Fasting insulin (mU/liter)	6.9 (4.1–12.4)	5.3 (3.9–8.0)	4.9 (3.3–7.8)	3.8%, $P < 0.001$[b]	0.9%, $P = 0.07$[b]	0.8%, $P = 0.1$[b]
Fasting glucose (mmol/liter)	4.9 (4.7–5.1)	4.9 (4.7–5.0)	4.8 (4.6–5.0)	0.3%, $P = 0.4$	0.0%, $P = 0.9$	0.0%, $P = 0.9$
SHBG (nmol/liter)	60.7 (37.7–76.3)	69.3 (54.8–86.7)	72.7 (55.1–93.3)	2.2%, $P = 0.008$	0.0%, $P = 0.9$	0.0%, $P = 0.9$
IGFBP-1 (ng/ml)	47.8 (34.9–69.4)	55.3 (46.9–70.7)	62.6 (49.7–77.2)	5.1%, $P < 0.001$	0.2%, $P = 0.4$	0.5%, $P = 0.2$
Leptin (mmol/liter)	6.2 (3.6–13.4)	5.6 (3.6–8.3)	4.4 (3.1–6.9)	5.3%, $P < 0.001$[b]	0.0%, $P = 0.8$[b]	0.2%, $P = 0.4$[b]

Data are expressed as median (interquartile range) of hormone levels by age-at-menarche group. *P* values represent tests of the trends across the three age-at-menarche groups.

[a] Variance (R-square) in age at menarche explained by hormone levels at age 8 yr. All models are adjusted for age at 8 yr.

[b] Based on log-transformed hormone values.

[c] Excluding 30 girls with self-reported pubertal breast development at baseline.

2). Results were similar when excluding the 30 girls with self-reported pubertal breast development. In a multivariable model, which included levels of IGF-I and adrenal androgens, BMI, and height, both IGF-I ($P = 0.04$) and androstenedione ($P = 0.06$) independently predicted earlier menarche. In sensitivity analyses, the two groups who attained menarche later (*i.e.* 12–13 *vs.* 13+ yr) also showed differences in IGF-I levels at age 8 yr ($P = 0.02$, adjusted for BMI and height).

Discussion

In a longitudinal population-based study, higher levels of IGF-I and adrenal androgens at age 8 yr were associated with earlier menarche, independent of BMI and height, suggesting that these hormones might directly promote a more rapid timing of pubertal development. Alternatively, these associations may reflect common early-life programming events that determine IGF-I levels, the timing of adrenarche, and the timing of puberty.

Reported associations between larger body size and BMI with early puberty and menarche have led to speculation of a direct role of childhood obesity on the secular trends in pubertal timing (5, 6). We found that body size (weight and height) and markers of body fat (BMI and waist circumference) at age 8 yr were strongly associated with earlier menarche. Some of the childhood hormonal alterations observed in girls who attained menarche earlier may therefore merely reflect these changes in body size and body composition, and we therefore adjusted for baseline height and BMI.

The GH/IGF-I axis has been causally implicated in pubertal timing because delayed puberty is a feature of rare human disorders of underactivity in this axis (1). In animal experimental models, intraventricular administration of IGF-I or IGF-I antibodies alters the timing of pubertal onset in rodents, and an increase in GnRH expression *in vitro* in the presence IGF-I and the activation of GH/IGF-I axis before the onset of pulsatile gonadotropin secretion in agonadal monkeys demonstrate a casual role of the GH/IGF-I axis in pubertal timing (7, 8). Our finding that circulating IGF-I levels are an independent predictor of early menarche is consistent with a reported association with IGF-I gene polymorphisms and suggests a direct role of IGF-I in pubertal timing in humans (9). IGF-I may modulate the reproductive system through widespread effects on hypothalamus, pituitary, and ovaries by its endocrine, paracrine, and autocrine actions based on the developmental and hormonal state (10). Furthermore, IGF-I plays a role in ovarian follicular formation and enhances FSH-mediated steroidogenesis (9).

Traditionally, adrenarche and gonadarche are considered to be separate processes; however, studies of girls with congenital adrenal hyperplasia or premature adrenarche suggest a direct role of adrenal androgens in initiating puberty in those disorders (11). Our findings indicate a possible role of adrenal androgens in pubertal timing in healthy children. Although the mechanisms are not certain, circumstantial evidence suggests that adrenal androgens may potentially reduce the inhibitory feedback from gonadal steroids, leading to increased GnRH pulsatility (11). Androstenedione is secreted from the ovary as well as from the adrenal gland, and consequently, its levels are higher in girls than in boys after age 8 yr (12). Hence, ovarian androgens may also play a role in pubertal timing in girls.

All of the other hormones we studied were also strongly associated with menarche timing, but these associations were attenuated after adjustment for baseline body size. Although such hormones could therefore be only "passive markers" of larger body size, it is still possible that some of these hormones might actively mediate the effects of larger body size on earlier menarche. Leptin, an adipocyte-derived hormone closely correlated with fat mass, has been implicated in signaling to the hypothalamus about body weight and energy balance. Although, initial observations suggested a direct role of leptin in inducing puberty, subsequently further data from animal studies, human observations of a steady rise with age rather than any rapid surge in levels before puberty, and reports that recombinant leptin treatment increases GnRH pulsatility in older, but not in younger, leptin-deficient children together suggest only a permissive role of leptin in regulating pubertal timing (1, 2).

Insulin resistance and subsequent hyperinsulinemia associated with childhood obesity are thought to play a mediating role in the development of early puberty (5). Increased insulin levels reduce levels of SHBG and IGFBP-1, which in turn leads to greater bioavailability of sex steroids and IGF-I, respectively (13, 14). In addition to the findings from observational studies, a causal role of insulin in puberty timing is supported by randomized controlled trials of metformin, which reduced the circulating hyperinsulinemia and prevented early pubertal onset and early menarche in girls with premature pubarche (15).

Recently reported associations of thinness at birth and early catch-up weight gain with earlier menarche (16, 17) suggest a key role of developmental programming resulting from prenatal and early postnatal environmental exposures in influencing the future tempo of growth and pubertal development. Higher levels of IGF-I at age 5 yr are seen in those children with lower birth weights and faster infancy weight gain, even independent of their current body composition (18). Similarly, both low birth weight and early postnatal catch-up weight gain are also associated with higher levels of adrenal androgens at age 8 yr, independent of body size (19, 20). Our findings now link these childhood hormonal alterations with early menarche and suggest that these hormones are involved in the developmental programming pathways that influence childhood growth and pubertal development. Alternatively, these hormonal changes may simply represent markers of common developmental pathways, and further evidence from intervention studies or genetic causal modeling is needed to confirm the causal relationships.

It is possible that higher IGF-I and adrenal androgen levels were simply markers of those girls who were already in early puberty at age 8 yr. However, our findings were unchanged after excluding those with self-reported breast development, and we also found differences in IGF-I levels at age 8 yr between the two groups of girls attaining menarche at 12–13 *vs.* 13+, at least 4 yr later, suggesting that these changes are not simply due to early pubertal activation at age 8 yr. Further studies of hormone levels at younger ages, possibly using ultrasensitive assays, would help to distinguish the effects of prepubertal hormone levels on the onset and progression of puberty.

In conclusion, in a longitudinal population-based study, we found that higher circulating levels of IGF-I and adrenal androgens at age 8 yr were related to earlier menarche, independent of body size. These findings support direct roles of these hormones on the regulation of pubertal maturation.

Acknowledgments

We are extremely grateful to all participants and their families, the midwives for their help in recruitment, and the research teams of the Avon Longitudinal Study of Parents and Children (ALSPAC). We also thank the National Institute for Health Research Biomedical Research Centre for their support and the Wellcome Trust for supporting the ALSPAC "Before-Breakfast Study" through a project grant.

Address all correspondence and requests for reprints to: Prof. David B. Dunger, University Department of Paediatrics, University of Cambridge, Box 116, Level 8, Addenbrooke's Hospital, Hills Road, Cambridge CB2 0QQ, United Kingdom. E-mail: dbd25@cam.ac.uk.

This work was supported by the Wellcome Trust through a project grant.

Disclosure Summary: The authors have nothing to declare.

References

1. **Veldhuis JD, Roemmich JN, Richmond EJ, Bowers CY** 2006 Somatotropic and gonadotropic axes linkages in infancy, childhood, and the puberty-adult transition. Endocr Rev 27:101–140
2. **Dunger DB, Ahmed ML, Ong KK** 2006 Early and late weight gain and the timing of puberty. Mol Cell Endocrinol 254–255:140–145
3. **Golding J, Pembrey M, Jones R** 2001 ALSPAC—the Avon Longitudinal Study of Parents and Children. I. Study methodology. Paediatr Perinat Epidemiol 15:74–87
4. **Christensen KY, Maisonet M, Rubin C, Flanders WD, Drews-Botsch C, Dominguez C, McGeehin MA, Marcus M** 2010 Characterization of the correlation between ages at entry into breast and pubic hair development. Ann Epidemiol 20:405–408
5. **Ahmed ML, Ong KK, Dunger DB** 2009 Childhood obesity and the timing of puberty. Trends Endocrinol Metab 20:237–242
6. **Ong KK, Ahmed ML, Dunger DB** 2006 Lessons from large population studies on timing and tempo of puberty (secular trends and relation to body size): the European trend. Mol Cell Endocrinol 254–255:8–12
7. **Suter KJ, Pohl CR, Wilson ME** 2000 Circulating concentrations of nocturnal leptin, growth hormone, and insulin-like growth factor-I

increase before the onset of puberty in agonadal male monkeys: potential signals for the initiation of puberty. J Clin Endocrinol Metab 85:808–814
8. **DiVall SA, Radovick S** 2008 Pubertal development and menarche. Ann NY Acad Sci 1135:19–28
9. **Zhao J, Xiong DH, Guo Y, Yang TL, Recker RR, Deng HW** 2007 Polymorphism in the insulin-like growth factor 1 gene is associated with age at menarche in Caucasian females. Hum Reprod 22:1789–1794
10. **Daftary SS, Gore AC** 2005 IGF-1 in the brain as a regulator of reproductive neuroendocrine function. Exp Biol Med (Maywood) 230:292–306
11. **Nader S** 2007 Adrenarche and polycystic ovary syndrome: a tale of two hypotheses. J Pediatr Adolesc Gynecol 20:353–360
12. **Tung YC, Lee JS, Tsai WY, Hsiao PH** 2004 Physiological changes of adrenal androgens in childhood. J Formos Med Assoc 103:921–924
13. **Holly JM, Smith CP, Dunger DB, Edge JA, Biddlecombe RA, Williams AJ, Howell R, Chard T, Savage MO, Rees LH, et al** 1989 Levels of the small insulin-like growth factor-binding protein are strongly related to those of insulin in prepubertal and pubertal children but only weakly so after puberty. J Endocrinol 121:383–387
14. **Caprio S** 1999 Insulin: the other anabolic hormone of puberty. Acta Paediatr Suppl 88:84–87
15. **Ibáñez L, Ong K, Valls C, Marcos MV, Dunger DB, de Zegher F** 2006 Metformin treatment to prevent early puberty in girls with precocious pubarche. J Clin Endocrinol Metab 91:2888–2891
16. **Ong KK, Emmett P, Northstone K, Golding J, Rogers I, Ness AR, Wells JC, Dunger DB** 2009 Infancy weight gain predicts childhood body fat and age at menarche in girls. J Clin Endocrinol Metab 94:1527–1532
17. **Adair LS** 2001 Size at birth predicts age at menarche. Pediatrics 107:E59
18. **Ong K, Kratzsch J, Kiess W, Dunger D** 2002 Circulating IGF-I levels in childhood are related to both current body composition and early postnatal growth rate. J Clin Endocrinol Metab 87:1041–1044
19. **Ong KK, Potau N, Petry CJ, Jones R, Ness AR, Honour JW, de Zegher F, Ibáñez L, Dunger DB** 2004 Opposing influences of prenatal and postnatal weight gain on adrenarche in normal boys and girls. J Clin Endocrinol Metab 89:2647–2651
20. **Ibáñez L, de Zegher F, Potau N** 1998 Premature pubarche, ovarian hyperandrogenism, hyperinsulinism and the polycystic ovary syndrome: from a complex constellation to a simple sequence of prenatal onset. J Endocrinol Invest 21:558–566