Blood Erythrocyte and Hemoglobin Concentrations in Premature Adrenarche

Pauliina Utriainen, Jarmo Jääskeläinen, and Raimo Voutilainen

Department of Pediatrics, University of Eastern Finland and Kuopio University Hospital, FI-70211 Kuopio, Finland

Context: Premature adrenarche (PA) is characterized by an earlier than normal increase in adrenocortical androgen production, and it is associated with increased serum IGF-I concentrations. Both the GH-IGF system and androgens, particularly testosterone, are known to enhance erythropoiesis.

Objective: Our objective was to test the hypothesis that blood erythrocyte count and blood hemoglobin (B-Hb) concentration are increased in PA.

Design, Participants, and Setting: Sixty-four prepubertal children (10 boys) with clinically and biochemically defined PA and 62 healthy prepubertal controls (10 boys) participating in our Premature Adrenarche study were examined, and a fasting blood sample was drawn at a university hospital.

Main Outcome Measures: We evaluated B-Hb and erythrocyte, thrombocyte, and leukocyte counts and their association with serum dehydroepiandrosterone sulfate (DHEAS), testosterone and IGF-I concentrations.

Results: Children with PA had higher mean blood erythrocyte count (4.74 *vs.* 4.64 $\times$ 10^{12}/liter, $P = 0.04$; significant difference in girls but not in boys) and a tendency toward higher B-Hb (130 *vs.* 128 g/liter, $P = 0.06$) than their controls. No difference was observed in leukocyte or thrombocyte counts between the study groups. In linear regression models including age, sex, body mass index SD score, IGF-I, and DHEAS or testosterone, B-Hb was associated with serum DHEAS ($P = 0.04$), testosterone ($P = 0.01$), and IGF-I ($P \leq 0.003$) concentrations in the entire study cohort and with IGF-I separately in girls ($P \leq 0.02$). Similar models showed a significant association of blood erythrocyte count with serum IGF-I concentration ($P = 0.003$–0.049) but not with DHEAS or testosterone.

Conclusions: Increased erythrocyte count in PA girls suggests that relatively small increases in serum androgen or IGF-I concentrations during adrenarche may associate with enhanced erythropoiesis. (***J Clin Endocrinol Metab* 98: E87–E91, 2013**)

Premature adrenarche (PA), the earlier than normal increase in adrenal androgen production in mid-childhood, has been associated with overweight, enhanced prepubertal growth in height, and increased bone mass (1–5). These findings suggest that excess of adrenal androgens during premature adrenarche has a growth-promoting effect.

The effect of androgens is mediated through the androgen receptor (AR). Adrenal androgens, mainly dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS), are weak AR agonist, but can be converted to AR-activating testosterone and dihydrotestosterone in peripheral tissues. AR is expressed also in bone marrow erythroblasts (6), and testosterone promotes erythropoiesis (7, 8). Men have higher blood erythrocyte counts and blood hemoglobin (B-Hb) concentrations than women, and the increase in B-Hb during puberty in boys is associated with rising testosterone concentrations (9, 10).

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Abbreviations: AR, Androgen receptor; B-Hb, blood hemoglobin; BMI, body mass index; CV, coefficient of variation; DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate; PA, premature adrenarche.

TABLE 1. Anthropometric, hormonal and blood count parameters in prepubertal children with PA (n = 64) and in prepubertal control children (n = 62)

	PA			Controls			
	All PA (n = 64)	PA girls (n = 54)	PA boys (n = 10)	All controls (n = 62)	Control girls (n = 52)	Control boys (n = 10)	*P*
Characteristics							
Age (yr)	7.6 (4.8–9.9)	7.6 (4.8–8.9)	7.2 (6.1–9.9)	7.5 (5.1–8.9)	7.5 (5.1–8.6)	6.6 (5.5–8.9)	0.13[c]
Height SDS	1.2 (−1.3–4.2)	1.2 (−1.3–4.2)[e]	1.1 (−1.2–3.4)	0.1 (−2.9–2.3)	0.0 (−2.9–2.1)	0.6 (−0.3–2.3)	<0.01[c]
BMI SDS	0.9 (−1.2–3.6)	0.8 (−1.2–3.1)[e]	1.5 (0.0–3.6)	−0.1 (−2.1–2.9)	−1.1 (−2.1–2.8)	0.8 (−1.1–2.9)	<0.01[d]
Serum concentrations							
DHEAS (μmol/liter)	2.1 (1.0–7.7)	2.1 (1.0–7.7)[e]	1.6 (1.0–4.3)	0.6 (0.1–0.9)	0.6 (0.2–0.9)	0.6 (0.1–0.7)	<0.01[d]
DHEA (nmol/liter)	7.6 (2.9–22.4)	7.8 (3.9–22.4)[e]	6.3 (2.9–16.4)	3.2 (1.2–10.2)	3.3 (1.2–10.2)	3.2 (2.3–7.1)	<0.01[d]
Andro (nmol/liter)	3.0 (0.7–6.6)	3.1 (0.7–6.6)[e]	2.1 (0.8–4.6)	1.0 (0.7–3.3)	1.0 (0.5–3.3)	1.0 (0.5–1.9)	<0.01[d]
SHBG (nmol/liter)	68 (18–187)	66 (18–176)[e]	92 (34–187)	108 (49–175)	108 (50–169)	98 (49–175)	<0.01[d]
IGF-I (nmol/liter)	24 (10–56)	24 (10–56)[e]	21 (10–45)	19 (8–42)	19 (8–35)	22 (9–42)	<0.01[d]
Testo (nmol/liter)[a]	0.42 (<0.35–1.17)	0.43 (<0.35–1.17)[e]	0.36 (<0.35–0.67)	<0.35 (<0.35–1.37)	<0.35 (<0.35–1.37)	<0.35 (<0.35–0.42)	<0.01[d]
Blood count							
Erythrocytes ($\times 10^{12}$/liter)[b]	4.74 (4.68–4.80)	4.74 (4.68–4.80)[f]	4.74 (4.48–5.00)	4.64 (4.57–4.71)	4.62 (4.55–4.70)	4.75 (4.50–5.00)	0.04[c]
Leukocytes ($\times 10^{9}$/liter)[a]	5.5 (4.9–6.3)	5.6 (4.8–6.6)	5.2 (5.0–5.7)	5.7 (5.0–6.6)	5.7 (5.0–6.6)	6.0 (4.7–7.0)	0.54[d]
Thrombocytes ($\times 10^{9}$/liter)[b]	295 (279–311)	297 (279–316)	282 (251–313)	300 (284–316)	303 (289–317)	286 (205–366)	0.63[c]
B-Hb (g/liter)[b]	130 (128–132)	130 (128–132)	131 (127–134)	128 (126–130)	128 (126–130)	128 (123–134)	0.06[c]
MCH (pg)[a]	28 (27–28)	28 (27–28)	28 (26–29)	28 (27–28)	28 (27–28)	27 (27–28)	0.47[d]
MCV (fl)[a]	81 (79–82)	81 (80–82)	81 (78–83)	81 (78–84)	81 (79–84)	79 (78–80)	0.72[d]

Unless indicated otherwise, characteristics and serum concentrations are shown as median (range). The *P* value represents comparison between All PA and All controls. Andro, Androstenedione; SDS, SD score; Testo, testosterone; MCH, erythrocyte mean hemoglobin; MCV, erythrocyte mean volume.

[a] Median (interquartile range).

[b] Mean (95% confidence interval).

[c] Student's *t* test.

[d] Mann-Whitney *U* test.

[e] $P < 0.001$ between PA and control girls.

[f] $P = 0.01$ between PA and control girls.

We hypothesized that prepubertal children with PA have higher B-Hb or erythrocyte counts than their prepubertal peers, whose androgen levels are physiologically low.

Subjects and Methods

The study group comprised 64 children with clinically (adrenarcheal signs before the age of 8 yr in girls or 9 yr in boys) and biochemically (serum DHEAS ≥1 μmol/liter) proven PA and 62 healthy prepubertal children without any signs of adrenarche and serum DHEAS below 1 μmol/liter, all participating in our Premature Adrenarche study project. The recruitment and inclusion criteria have been described in detail previously (1, 5, 11). At examination, girls had to be less than 9 yr and boys less than 10 yr of age. Children with central puberty, any endocrine disorder, or long-term medication were excluded. All children had normal serum 17-hydroxyprogesterone concentration excluding 21-hydroxylase deficiency, and none presented with an adrenal tumor. Height was measured with a Harpenden stadiometer and converted to SD scores according to the Finnish growth charts as described (1). Weight was measured after an overnight fast, and body mass index (BMI) was calculated and converted to BMI SD scores according to British reference values as described (1). An iv cannula was placed for blood sampling. A blood sample for the measurements of B-Hb and erythrocyte, leukocyte, and throm-

TABLE 2. Linear regression model for hemoglobin and erythrocyte count in the whole study group and separately in girls

	All subjects (n = 126)					
	Hemoglobin (g/liter)			Erythrocyte count ($\times 10^{12}$/liter)		
	Standardized coefficient	Regression coefficient (95% CI)	*P*	Standardized coefficient	Regression coefficient (95% CI)	*P*
Age	0.008	0.007 (−1.27–1.40)	0.92	−0.025	−0.007 (−0.06–0.04)	0.78
Gender (boys vs. girls)	0.068	1.28 (−1.93–4.49)	0.43	0.087	0.062 (−0.06–0.19)	0.32
DHEAS (μmol/liter)	0.193	1.08 (0.073–2.08)	0.04	0.111	0.023 (−0.015–0.062)	0.23
IGF-I (nmol/liter)	0.32	0.26 (0.11–0.41)	0.001	0.279	0.009 (0.003–0.014)	0.003
BMI SDS	−0.051	−0.29 (−1.33–0.76)	0.59	0.073	0.019 (−0.02–0.059)	0.45
R^2 of the model			0.123			0.100

CI, Confidence interval; SDS, SD score; R^2, estimated size of the effect of all variables in the model.

bocyte counts, erythrocyte mean cellular volume and hemoglobin, and serum IGF-I, SHBG, testosterone, DHEA, DHEAS, and androstenedione concentrations was drawn between 0900 and 1000 h after an overnight fast. Blood erythrocyte, thrombocyte, and leukocyte counts were analyzed by automated electronic cell counting and B-Hb by a cyanmethemoglobin method. Serum SHBG [intra- and interassay coefficients of variation (CV) of 4.0 and 2.6%, respectively] was determined by a time-resolved fluoroimmunoassay (AutoDelfia; PerkinElmer Life and Analytical Sciences Wallac Oy, Turku, Finland). DHEAS (CV = 3.8–5.3 and 6.3–11.0%, respectively), androstenedione (3.2–9.4 and 4.1–15.6%), and testosterone (<10.0 and <13.0%) were determined by specific RIAs, and IGF-I by an immunochemiluminometric assay using an Immulite 2000 analyzer (interassay CV 4% and total CV 9%) (all from Diagnostic Products Corp., Los Angeles, CA). Serum DHEA concentrations were measured with an in-house RIA derived from a previously described method (intra- and interassay CV of 8 and 12–13%, respectively) (12). The study protocol was approved by the Research Ethics Committee of Kuopio University Hospital. Informed written consent from parents and assent from the children were obtained for participation in the study.

Statistical analyses

SPSS version 17.0 was used for the statistical analyzes. All parameters were first tested for normality. The differences in blood characteristics between the study groups were analyzed with the independent-samples *t* or Mann-Whitney *U* test according to the distribution of the variable. The means and 95% confidence intervals are shown for the normally distributed parameters (B-Hb and erythrocyte and thrombocyte counts) and medians with interquartile ranges for the nonnormally distributed parameters. The previously reported group characteristics are shown as median and range, and they were tested with the *t* (age and height) or Mann-Whitney *U* test (BMI SD score, SHBG, DHEAS, DHEA, androstenedione, and IGF-I) depending on the distribution of the variable. Because serum testosterone concentration was below the detection limit of 0.35 nmol/liter in many subjects, the children were stratified into three groups by serum testosterone: 1) less than or equal to 0.35 nmol/liter (below the detection limit, two lowest quartiles), 2) 0.36–0.47 nmol/liter (3rd quartile), and 3) more than 0.47 nmol/liter (highest quartile). This stratification was used in further analyses. Differences in other biochemical parameters between the three testosterone groups were analyzed using ANOVA with Bonferroni correction. Covariance analyses were used to examine the age- and sex-adjusted group differences in hematological variables. Possible linear correlations between blood count variables and other parameters were examined using Pearson's correlation test and linear regression models. An interaction between sex and androgens (DHEAS and testosterone group) was also analyzed in the multivariate models.

Results

The children with PA had a higher mean blood erythrocyte count than the control subjects (4.74 *vs.* 4.64 × 10^{12}/liter, $P = 0.037$), and there was a tendency toward higher mean B-Hb concentration (130 *vs.* 128 g/liter, $P = 0.063$) (Table 1). The difference in erythrocyte count was observed between the PA and control girls (4.74 *vs.* 4.62 × 10^{12}/liter, $P = 0.007$) but not between the PA and control boys (4.74 *vs.* 4.75 × 10^{12}/liter, $P = 1.0$). The difference in erythrocyte count between the PA and control groups remained significant when adjusted for age and sex ($P = 0.039$) and between the PA and control girls after age adjustment ($P = 0.014$). There were no significant differences in other blood count variables between the PA and control groups (Table 1).

The serum sex hormone concentrations of the study groups (Table 1) have partly been reported previously (1, 5, 11). In the PA subjects, B-Hb was correlated with serum DHEAS (r = 0.31, $P = 0.014$) and height SD score (r = 0.30, $P = 0.017$), and both B-Hb and erythrocyte count with IGF-I (r = 0.40, $P = 0.001$ and r = 0.30, $P = 0.017$, respectively). In the controls, serum IGF-I was correlated with erythrocyte count (r = 0.30, $P = 0.020$). In the whole study population, IGF-I correlated with DHEAS, DHEA, and androstenedione (r = 0.25–0.30, $P = 0.001$–0.005). Testosterone was below the detection limit (0.35 nmol/liter) in 51% (63 of 124) of all subjects (33% of the PA and 68% of the controls). Within the entire study population, the subjects with

TABLE 2. Continued

	Girls (n = 106)					
	Hemoglobin (g/liter)			Erythrocytes (×10^{12}/liter)		
	Standardized coefficient	Regression coefficient (95% CI)	*P*	Standardized coefficient	Regression coefficient (95% CI)	*P*
Age	0.051	0.44 (−1.19–2.06)	0.59	−0.011	−0.00 (−0.06–0.05)	0.91
Gender (boys vs. girls)	0.184	1.05 (−0.06–2.15)	0.063	0.162	0.03 (−0.01–0.07)	0.10
DHEAS (μmol/liter)	0.302	0.25 (0.08–0.42)	0.004	0.231	0.01 (0.00–0.01)	0.03
IGF-I (nmol/liter)	−0.034	−0.19 (−1.34–0.96)	0.74	0.147	0.03 (−0.01–0.07)	0.15
BMI SDS			0.124			0.125
R^2 of the model						

their testosterone in the highest quartile had higher B-Hb ($P = 0.002$), DHEAS ($P < 0.001$), DHEA ($P < 0.001$), androstenedione ($P < 0.001$), and IGF-I ($P = 0.001$) than those in the lowest testosterone group.

In the multivariate linear regression models, B-Hb was associated independently with serum IGF-I and DHEAS in the entire study cohort (Table 2) and with IGF-I separately in the girls (Table 2), whereas blood erythrocyte count was associated only with serum IGF-I in the entire study group and separately in the girls (Table 2). When testosterone (categorized into three groups) was included (instead of DHEAS) in the otherwise similar models, B-Hb was independently associated with serum IGF-I and testosterone in the entire study cohort (standardized coefficient $\beta = 0.28$, $P = 0.003$ for IGF-I; and $\beta = 0.24$, $P = 0.013$ for testosterone) and separately in girls ($\beta = 0.25$, $P = 0.018$; and $\beta = 0.26$, $P = 0.013$, respectively). In this regression model, erythrocyte count was associated with IGF-I in the entire group (0.28, $P = 0.005$) and separately in girls (0.21, $P = 0.049$). In the multivariate models for erythrocyte count, there was a significant interaction between testosterone and sex in the PA ($P = 0.037$) and the entire study group ($P = 0.05$).

Discussion

In the present study, the girls with PA had higher mean blood erythrocyte count than the control girls. Longitudinal studies on adolescent boys during normal and delayed puberty indicate that the pubertal increase in B-Hb is caused by androgens, mainly testosterone (9, 10). In adult men, testosterone increases whereas deprivation of testosterone decreases B-Hb (13, 14). The erythropoiesis-stimulating effect of testosterone has also been shown *in vitro* (7, 8). Our study suggests that even a relatively small increase in adrenal DHEA(S) during adrenarche could influence erythropoiesis. The suggested effect of DHEA(S) on erythropoiesis is most likely mediated via peripheral conversion to testosterone. A direct effect of DHEA via sex hormone receptors is also theoretically possible, but that would probably require much higher DHEA concentrations (15). We were not able to find any reports on B-Hb or erythrocyte count in other hyperandrogenic conditions like congenital adrenal hyperplasia in children.

B-Hb and erythrocyte count were independently associated with IGF-I in our prepubertal subjects, the associations being stronger than those with DHEAS or testosterone. This could indicate that the effect of (adrenal) androgens on erythropoiesis is mediated at least partly via IGF-I. Indeed, a similar interplay between androgens and IGF-I in erythropoiesis has been suggested in patients with chronic renal anemia receiving erythropoietin or androgen treatment (16). Because we did not measure serum erythropoietin concentrations, we can only speculate on the possible interplay between androgens and GH-IGF-I with erythropoietin in PA subjects. However, the role of the GH-IGF-I axis in erythropoiesis is well documented in children during puberty and GH treatment (17, 18).

We have previously shown that our prepubertal children with PA have enhanced prepubertal growth, higher circulating IGF-I concentrations (1), and higher bone mineral density (determined by increased body size) (5) compared with their prepubertal healthy peers. The positive effect of adrenal androgens on bone strength has also been found in normally timed adrenarche (19). The present and previous findings suggest that the PA children having increased IGF-I concentrations and enhanced growth (1) may also have enhanced erythropoiesis. This is interesting given that the ultimate role of adrenarche remains unclear. Different study designs are needed to investigate whether normally timed adrenarche has similar effects on hematological variables as seen in our PA girls. Indeed, in one population-based study, DHEAS associated with B-Hb in healthy adolescent boys (20).

The tendency toward higher B-Hb in the PA than control girls was associated with higher testosterone (or DHEAS) and IGF-I levels in the PA girls. Because neither IGF-I nor testosterone levels of the PA boys were significantly increased compared with the control boys or PA girls, it is not surprising that the mean B-Hb concentration in the prepubertal PA boys was not higher than in the PA girls. Due to the small number of boys, there is not enough power to reach statistical significance in the subgroup of boys. Although BMI SD score had no independent association with B-Hb or erythrocyte count in our linear regression models, we cannot exclude the possibility that higher weight in PA than control children could have some effect on blood count variables.

To conclude, prepubertal girls with PA showed mildly increased blood erythrocyte count compared with their healthy peers. Serum IGF-I, DHEAS, and testosterone concentrations were associated with B-Hb levels in our prepubertal subjects. These findings suggest that the relatively small increases in androgen concentrations during adrenarche may be able to stimulate erythropoiesis, and IGF-I could be involved in this effect.

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Address all correspondence and requests for reprints to: Pauliina Utriainen, Department of Pediatrics, Kuopio University Hospital and University of Eastern Finland, P.O. Box 1777, FI-70211 Kuopio, Finland. E-mail: pauliina.utriainen@uef.fi.

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References

1. **Utriainen P, Voutilainen R, Jääskeläinen J** 2009 Girls with premature adrenarche have accelerated early childhood growth. J Pediatr 154:882–887
2. **Idkowiak J, Lavery GG, Dhir V, Barrett TG, Stewart PM, Krone N, Arlt W** 2011 Premature adrenarche: novel lessons from early onset androgen excess. Eur J Endocrinol 165:189–207
3. **Ibáñez L, Potau N, Ong K, Dunger DB, De Zegher F** 2000 Increased bone mineral density and serum leptin in non-obese girls with precocious pubarche: relation to low birthweight and hyperinsulinism. Horm Res 54:192–197
4. **Sopher AB, Thornton JC, Silfen ME, Manibo A, Oberfield SE, Wang J, Pierson Jr RN, Levine LS, Horlick M** 2001 Prepubertal girls with premature adrenarche have greater bone mineral content and density than controls. J Clin Endocrinol Metab 86:5269–5272
5. **Utriainen P, Jääskeläinen J, Saarinen A, Vanninen E, Mäkitie O, Voutilainen R** 2009 Body composition and bone mineral density in children with premature adrenarche and the association of *LRP5* gene polymorphisms with bone mineral density. J Clin Endocrinol Metab 94:4144–4151
6. **Claustres M, Sultan C** 1988 Androgen and erythropoiesis: evidence for an androgen receptor in erythroblasts from human bone marrow cultures. Horm Res 29:17–22
7. **Moriyama Y, Fisher JW** 1975 Effects of testosterone and erythropoietin on erythroid colony formation in human bone marrow cultures. Blood 45:665–670
8. **Udupa KB, Crabtree HM, Lipschitz DA** 1986 In vitro culture of proerythroblasts: characterization of proliferative response to erythropoietin and steroids. Br J Haematol 62:705–714
9. **Thomsen K, Riis B, Krabbe S, Christiansen C** 1986 Testosterone regulates the haemoglobin concentration in male puberty. Acta Paediatr Scand 75:793–796
10. **Hero M, Wickman S, Hanhijärvi R, Siimes MA, Dunkel L** 2005 Pubertal upregulation of erythropoiesis in boys is determined primarily by androgen. J Pediatr 146:245–252
11. **Utriainen P, Voutilainen R, Jääskeläinen J** 2009 Continuum of phenotypes and sympathoadrenal function in premature adrenarche. Eur J Endocrinol 160:657–665
12. **Apter D, Pakarinen A, Hammond GL, Vihko R** 1979 Adrenocortical function in puberty: serum ACTH, cortisol and dehydroepiandrosterone in girls and boys. Acta Paediatr Scand 68:599–604
13. **Weber JP, Walsh PC, Peters CA, Spivak JL** 1991 Effect of reversible androgen deprivation on hemoglobin and serum immunoreactive erythropoietin in men. Am J Hematol 36:190–194
14. **Coviello AD, Kaplan B, Lakshman KM, Chen T, Singh AB, Bhasin S** 2008 Effects of graded doses of testosterone on erythropoiesis in healthy young and older men. J Clin Endocrinol Metab 93:914–919
15. **Chen F, Knecht K, Birzin E, Fisher J, Wilkinson H, Mojena M, Moreno CT, Schmidt A, Harada S, Freedman LP, Reszka AA** 2005 Direct agonist/antagonist functions of dehydroepiandrosterone. Endocrinology 146:4568–4576
16. **Navarro JF, Mora C, Macía M, García J** 2002 Randomized prospective comparison between erythropoietin and androgens in CAPD patients. Kidney Int 61:1537–1544
17. **Anttila R, Koistinen R, Seppälä M, Koistinen H, Siimes MA** 1994 Insulin-like growth factor I and insulin-like growth factor binding protein 3 as determinants of blood hemoglobin concentration in healthy subjects. Pediatr Res 36:745–748
18. **Vihervuori E, Virtanen M, Koistinen H, Koistinen R, Seppälä M, Siimes MA** 1996 Hemoglobin level is linked to growth hormone-dependent proteins in short children. Blood 87:2075–2081
19. **Remer T, Boye KR, Hartmann M, Neu CM, Schoenau E, Manz F, Wudy SA** 2003 Adrenarche and bone modeling and remodeling at the proximal radius: weak androgens make stronger cortical bone in healthy children. J Bone Miner Res 18:1539–1546
20. **Arquitt AB, Stoecker BJ, Hermann JS, Winterfeldt EA** 1991 Dehydroepiandrosterone sulfate, cholesterol, hemoglobin, and anthropometric measures related to growth in male adolescents. J Am Diet Assoc 91:575–579