

Obesity and Sex Steroid Changes across Puberty: Evidence for Marked Hyperandrogenemia in Pre- and Early Pubertal Obese Girls

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Context: Peripubertal obesity is associated with abnormal sex steroid concentrations, but the timing of onset and degree of these abnormalities remain unclear.

Objective: The objective of the study was to assess the degree of hyperandrogenemia across puberty in obese girls and assess overnight sex steroid changes in Tanner stage 1–3 girls.

Design: This was a cross-sectional analysis.

Setting: The study was conducted at general clinical research centers.

Subjects: Thirty normal-weight (body mass index for age < 85%) and 74 obese (body mass index for age \geq 95%) peripubertal girls.

Intervention: Blood samples (circa 0500–0700 h) were taken while fasting. Samples from the preceding evening (circa 2300 h) were obtained in 23 Tanner 1–3 girls.

Main Outcome Measures: Hormone concentrations stratified by Tanner stage were measured.

Results: Compared with normal-weight girls, mean free testosterone (T) was elevated 2- to 9-fold across puberty in obese girls, whereas fasting insulin was 3-fold elevated in obese Tanner 1–3 girls ($P < 0.05$). Mean LH was lower in obese Tanner 1 and 2 girls ($P < 0.05$) but not in more mature girls. In a subgroup of normal-weight Tanner 1–3 girls ($n = 17$), mean progesterone (P) and T increased overnight 2.3- and 2.4-fold, respectively ($P \leq 0.001$). In obese Tanner 1–3 girls ($n = 6$), evening P and T were elevated, and both tended to increase overnight [mean 1.4- and 1.6-fold, respectively ($P = 0.06$)].

Conclusions: Peripubertal obesity is associated with hyperandrogenemia and hyperinsulinemia throughout puberty, being especially marked shortly before and during early puberty. P and T concentrations in normal-weight Tanner 1–3 girls increase overnight, with similar but less evident changes in obese girls. (*J Clin Endocrinol Metab* 92: 430–436, 2007)

OBESITY IS ASSOCIATED with hyperandrogenemia (HA) in adult women (1), and a similar relationship has been described in late female adolescence (2–5). There are few data on the relationship between female obesity and HA in early puberty, a critical time when the hypothalamic-pituitary-ovarian interactions governing reproductive function are established. Recent reports have documented an association between obesity and testosterone (T) in pre- and early pubertal (Tanner breast stages 1–3) girls (6, 7). To delineate the timing and extent of this association across pubertal maturation, we assessed early-morning (fasting) hormone levels in a group of obese and normal weight peripubertal girls stratified by Tanner stage.

In addition to the rise of sex steroid concentrations across female pubertal development, both estradiol (E_2) and T exhibit diurnal changes, with nadirs occurring in the late evening hours and peaks between 0600 and 1000 h (8–11). However, there are no published data regarding diurnal changes in progesterone (P), and it is unknown whether obesity modifies the diurnal changes in sex steroids during puberty. Therefore, we evaluated overnight sex steroid changes in a subset of normal weight and obese pre- and early pubertal (Tanner stages 1–3) girls.

Subjects and Methods

Subjects

Our collaborative group collected hormonal and anthropomorphic data in girls across the pubertal spectrum (ages 7–17 yr) with varying degrees of adiposity. Building on previous observations (7), the present analysis was restricted to those girls who were either normal weight [gender-specific body mass index (BMI) for age < 85th percentile; $n = 30$] or obese (BMI for age \geq 95th percentile; $n = 74$). Girls who were at risk of overweight (*i.e.* BMI for age between 85th and 95th percentile) were not included in this analysis because they are generally felt to be at intermediate risk for obesity-related abnormalities.

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Abbreviations: BMI, Body mass index; CV, coefficient of variation; DHEAS, dehydroepiandrosterone sulfate; E_2 , estradiol; HA, hyperandrogenemia; HOMA, homeostatic model assessment; P, progesterone; PCOS, polycystic ovary syndrome; T, testosterone.

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These categories are consistent with those endorsed by the Centers for Disease Control and Prevention and the American Academy of Pediatrics. Exact BMI-for-age percentiles were calculated using a SAS program that incorporates normative data from the National Health Examination and Nutrition Examination Surveys (12) (available on the Centers for Disease Control and Prevention Web site, <http://www.cdc.gov/nccdphp/dnpa/growthcharts/sas.htm>). Volunteers were recruited through advertisements and from endocrinology clinics. Subjects were evaluated with morning fasting blood samples obtained at the University of Virginia Health System ($n = 49$), the University of Michigan ($n = 15$), the University of California at San Diego ($n = 7$), and Yale ($n = 33$). In older girls in whom cycle stage was unclear, a morning P concentration greater than 2 ng/ml was grounds for exclusion. Subjects included 6 and 12 Tanner stage 1; 7 and 23 Tanner 2; 6 and 16 Tanner 3; and 11 and 23 Tanner 4–5 normal weight and obese girls, respectively. Reported racial categories included white ($n = 83$), black ($n = 19$), Asian ($n = 1$), and other ($n = 1$). Seventeen of 104 were of Hispanic descent. Racial and ethnic classifications were balanced between normal-weight and obese groups. Some of these subjects were included in a previous report (7).

Study procedures

Study procedures were approved by the institutional review boards at each institution. Informed assent and consent were obtained from study participants and parents, respectively. Participants were taking no medications known to affect the reproductive axis, and none had used hormonal medications for 90 d before study.

Each volunteer underwent a detailed history and physical exam including assessment of pubertal stage using the Tanner scale for breast development (13). We did not use Tanner scales for hair growth.

An early morning (circa 0500–0700 h) blood sample was obtained for measurement of T, SHBG, LH, FSH, dehydroepiandrosterone sulfate (DHEAS), E_2 , P, and cortisol. Insulin and glucose were measured on the same blood sample when fasting for at least 6 h was confirmed ($n = 80$). Twenty-three pre- and early pubertal girls (six Tanner 1, five Tanner 2, and six Tanner 3 normal weight girls; and two obese girls in each of the first three Tanner stages) also had a sample drawn for T, E_2 , P, and cortisol the preceding evening (circa 2200–2300 h).

Hormonal measurements

Assays were performed by the Ligand Core Laboratory of the Center for Research in Reproduction at the University of Virginia Health System. Total T, E_2 , and P were measured by RIA (Diagnostic Products Corp., Los Angeles, CA; sensitivities 5.0 ng/dl, 10 pg/ml, and 0.05 ng/ml, respectively; intraassay coefficients of variation (CVs) 4.4–10, 5.2–6.8, and 2.9–5.0%, respectively; and interassay CVs 8.8–12.4, 11.8–15.8, and 9.1–10.9%, respectively).

Given the possibility of cross-reaction of cortisol in the P assay, we performed a series of studies in which P-free sera with various concentrations of cortisol were assessed using the P assay. Cortisol in concentrations of 2.5 and 25 $\mu\text{g}/\text{dl}$ measured as approximately 25 and 250 pg/ml in the P assay, respectively, with intermediate cortisol concentrations yielding intermediate P measurements. A cortisol concentration-P measurement nomogram was constructed, which allowed adjustment of measured P concentrations for the contribution of cortisol. Cortisol was measured using RIA (Diagnostic Products Corp.; sensitivity 1 $\mu\text{g}/\text{dl}$; intraassay CV 4.2–4.7%; interassay CV 6.5–8.1%).

SHBG and DHEAS were measured by chemiluminescence (Diagnostic Products Corp.; sensitivities 0.2 nmol/liter and 7 $\mu\text{g}/\text{dl}$; intraassay CVs 2.0–3.5 and 5.4–6.2%; and interassay CVs 3.8–5.7 and 6.8–7.8%, respectively). Insulin was measured by chemiluminescence (Diagnostic Products Corp.; sensitivity 2.6 $\mu\text{IU}/\text{ml}$; intraassay CV 3.0–4.0%; and interassay CV 8.3–8.8%, respectively) or RIAs (Diagnostic Systems Laboratories, Inc., Webster, TX; sensitivity 1.3 $\mu\text{IU}/\text{ml}$; intraassay CV 7.5–8.5%; and interassay CV 9.5–21%; or Linco Research, Inc., St. Charles, MO; intraassay CV 4.5%, interassay CV 10%). LH and FSH were measured by chemiluminescence (Diagnostic Products Corp.; sensitivities 0.1 and 0.05 IU/liter; intraassay CVs 2.3–4.1 and 2.2–2.6%; and interassay CVs 5.3–6.6 and 4.9–6.3%, respectively). Glucose was measured via glucose oxidase method using a glucose analyzer (Beckman

Instruments, Brea, CA). Samples with measured values below assay sensitivity were assigned the value of the assay's sensitivity.

Free T was calculated from total T and SHBG using the following equation: $FT = [T - (N)(FT)] / [(K_T)(SHBG) - (K_T)(T) + (N)(K_T)(FT)]$. In this equation, FT = free T (picomoles per liter); K_T = association constant of SHBG for T (1.0×10^9); T = total T concentration (nanograms per deciliter); SHBG = SHBG concentration (nanomoles per liter); and $N = (K_A)(C_A) + 1$, where K_A = association constant of albumin for T (3.6×10^4) and C_A = concentration of albumin (assumed to be 4.3 g/dl) (14).

Homeostatic model assessment (HOMA), an estimate of insulin resistance, was calculated from glucose (expressed as millimoles per liter) and insulin (expressed as micromoles per milliliter) using the equation (glucose \times insulin)/22.5 (15).

Statistical methods

Data are presented as mean \pm SEM unless otherwise noted. We used nonparametric statistical tests, which are based on ranks of observations and require no assumptions about the underlying distribution of data. All hypothesis tests were two sided.

Wilcoxon rank sum tests (*i.e.* two sample Wilcoxon tests) were used to compare parameters between normal-weight and obese girls within their respective Tanner stage grouping. The method of normal approximation was used for comparisons between normal-weight and obese Tanner stage 4–5 girls because there were at least 10 observations in each group. Exact tests were performed for all other pairwise comparisons, which involved less than 10 observations in the respective normal-weight groups. These tests were conducted at the 0.05 level of significance, but we secondarily applied a criterion for significance of 0.005 as guided by the highly conservative Bonferroni method of adjusting for multiple comparisons.

Wilcoxon sign rank tests were used to assess overnight changes in sex steroid values in the groups of normal-weight and obese Tanner 1–3 girls. Wilcoxon rank sum tests (*i.e.* two sample Wilcoxon tests) were used to evaluate for differences in sex steroid concentrations at 2300 h and 0600 h, respectively, between normal-weight and obese Tanner 1–3 girls. These tests were conducted at the 0.05 level of significance with no correction for multiple comparisons.

Results

Early-morning hormonal values stratified by Tanner stage

The obese girls in our cohort demonstrated significant hyperandrogenism throughout puberty, and this was especially marked in pre- and early pubertal girls (Table 1 and Fig. 1). Compared with normal-weight girls, mean total T was 4.5-fold (median 7.1-fold) higher in obese Tanner 1 (prepubertal) girls and 1.6- and 3.3-fold (median 3.2- and 2.8-fold) elevated in obese Tanner stage 2 and 3 girls, respectively ($P < 0.005$ for Tanner 1 and 3; $P = 0.08$ for Tanner 2). Additionally, mean SHBG was 59–69% (median 57–72%) lower in the obese Tanner 1, 2, and 3 girls ($P < 0.0001$ for Tanner 1; $P < 0.001$ for Tanner 2 and 3). Together these account for the estimated 8.8-, 2.2-, and 5.8-fold higher mean free T (17.5-, 5.0-, and 5.3-fold higher median free T) in obese Tanner stages 1, 2, and 3 girls, respectively ($P < 0.001$ for Tanner 1 and 3; $P < 0.05$ for Tanner 2). Similar differences were observed in the more mature, Tanner 4–5 girls.

To define Tanner stage-specific normal ranges for free T, we evaluated this parameter in normal-weight girls with no evidence of hirsutism (and no irregular periods in girls at least 2 yr after menarche). The maximum free T was 4.8 pmol/liter in Tanner 1, 11.0 pmol/liter in Tanner 2, 15.1 pmol/liter in Tanner 3, and 31.2 pmol/liter in Tanner 4–5 girls. The proportion of obese girls with free T values above the Tanner stage-specific normal range was between 60 and 94% (Fig. 2).

TABLE 1. Early morning hormonal data in normal weight and obese peripubertal girls stratified by Tanner stage

	Normal		Obese	
	n	Mean ± SEM (median)	n	Mean ± SEM (median)
Tanner 1				
Age (yr)	6	8.8 ± 0.3 (9.0)	12	9.0 ± 0.5 (8.6)
BMI (kg/m ²)	6	15.8 ± 0.5 (16.1)	12	31.3 ± 1.4 (30.8) ^d
BMI-for-age percentile	6	41.1 ± 9.0 (44.9)	12	99.2 ± 0.2 (99.4) ^d
Total T (pg/ml)	6	43 ± 13 (30)	12	192 ± 33 (213) ^b
SHBG (nmol/liter)	6	60.4 ± 5.3 (63.5)	12	18.8 ± 2.4 (20.0) ^d
Free T (pmol/liter)	6	1.9 ± 0.7 (1.1)	12	16.7 ± 2.9 (19.3) ^c
Fasting insulin (μIU/ml)	4	4.6 ± 1.0 (5.2)	10	32.3 ± 4.2 (33.5) ^b
Glucose (mg/dl)	4	94 ± 4 (95)	10	93 ± 2 (92)
HOMA	4	1.0 ± 0.2 (1.1)	10	7.5 ± 1.1 (7.7) ^b
LH (IU/liter)	6	0.2 ± 0.1 (0.1)	12	0.1 ± 0.03 (0.1) ^{b*}
FSH (IU/liter)	6	2.1 ± 0.6 (1.8)	5	0.5 ± 0.3 (0.3) ^{a*}
DHEAS (μg/dl)	6	20 ± 9 (9)	12	55 ± 9 (53) ^{a*}
E ₂ (pg/ml)	6	22 ± 7 (18)	5	15 ± 4 (22)
P (pg/ml)	4	166 ± 43 (165)	3	169 ± 64 (150)
Tanner 2				
Age (yr)	7	11.9 ± 0.4 (12.2)	23	9.8 ± 0.3 (9.7) ^b
BMI (kg/m ²)	7	17.2 ± 0.9 (16.9)	23	32.5 ± 1.2 (32.8) ^d
BMI-for-age percentile	7	35.3 ± 10.8 (28.8)	23	99.2 ± 0.2 (99.5) ^d
Total T (pg/ml)	7	184 ± 74 (100)	23	290 ± 30 (320)
SHBG (nmol/liter)	7	49.3 ± 7.5 (44.1)	23	20.0 ± 2.4 (18.9) ^c
Free T (pmol/liter)	7	11.6 ± 6.1 (4.7)	23	25.2 ± 3.1 (23.6) ^{a*}
Fasting insulin (μIU/ml)	4	13.6 ± 3.9 (11.8)	17	38.4 ± 7.6 (28.6)
Glucose (mg/dl)	4	102 ± 3 (104)	17	91 ± 2 (91)
HOMA	4	3.5 ± 1.1 (3.0)	17	8.7 ± 1.8 (6.4)
LH (IU/liter)	7	2.7 ± 1.5 (1.2)	21	0.6 ± 0.2 (0.1) ^{a*}
FSH (IU/liter)	6	3.0 ± 0.5 (2.6)	12	2.1 ± 0.8 (0.8)
DHEAS (μg/dl)	7	59 ± 18 (46)	20	78 ± 11 (66)
E ₂ (pg/ml)	6	28 ± 9 (27)	12	20 ± 5 (14)
P (pg/ml)	6	393 ± 107 (278)	6	376 ± 110 (285)
Tanner 3				
Age (yr)	6	12.6 ± 0.4 (13.0)	16	12.3 ± 0.3 (12.0)
BMI (kg/m ²)	6	18.6 ± 0.9 (18.5)	16	34.6 ± 1.6 (34.0) ^d
BMI-for-age percentile	6	48.4 ± 12.7 (44.5)	16	98.8 ± 0.3 (99.2) ^d
Total T (pg/ml)	6	150 ± 32 (159)	16	500 ± 75 (444) ^b
SHBG (nmol/liter)	6	49.2 ± 8.3 (48.1)	16	15.4 ± 2.7 (13.5) ^c
Free T (pmol/liter)	6	8.4 ± 2.3 (7.8)	16	49.1 ± 9.1 (41.5) ^d
Fasting insulin (μIU/ml)	4	10.3 ± 3.0 (8.2)	14	38.1 ± 4.2 (35.8) ^b
Glucose (mg/dl)	4	90 ± 5 (89)	14	92 ± 2 (92)
HOMA	4	2.4 ± 0.8 (1.7)	14	8.8 ± 1.1 (8.1) ^b
LH (IU/liter)	6	4.3 ± 0.9 (4.3)	16	3.0 ± 0.7 (2.5)
FSH (IU/liter)	6	5.2 ± 0.8 (4.9)	3	3.6 ± 0.9 (3.1)
DHEAS (μg/dl)	6	58 ± 14 (60)	16	105 ± 13 (91)
E ₂ (pg/ml)	6	29 ± 8 (35)	3	44 ± 20 (58)
P (pg/ml)	6	371 ± 17 (386)	2	1140 ± 599 (1140)
Tanner 4–5				
Age (yr)	11	13.8 ± 0.4 (13.4)	23	15.4 ± 0.3 (15.8) ^{a*}
Tanner stage	11	4.4 ± 0.2 (4)	23	4.8 ± 0.1 (5) ^{b*}
BMI (kg/m ²)	11	20.4 ± 0.6 (20.8)	23	37.8 ± 1.2 (37.4) ^d
BMI-for-age percentile	11	62.0 ± 5.1 (64.5)	23	98.5 ± 0.3 (99.1) ^d
Total T (pg/ml)	11	262 ± 33 (250)	23	440 ± 37 (430) ^{b*}
SHBG (nmol/liter)	11	34.2 ± 3.9 (27.8)	23	15.7 ± 1.5 (13.1) ^c
Free T (pmol/liter)	11	15.9 ± 1.9 (14.9)	23	41.1 ± 4.1 (39.0) ^d
Fasting insulin (μIU/ml)	11	13.3 ± 2.7 (11.6)	16	25.3 ± 1.9 (25.1) ^c
Glucose (mg/dl)	11	90 ± 3 (89)	16	85 ± 2 (85)
HOMA	11	3.0 ± 0.7 (2.4)	16	5.3 ± 0.4 (5.2) ^b
LH (IU/liter)	11	6.4 ± 0.7 (5.4)	23	5.9 ± 0.6 (5.3)
FSH (IU/liter)	11	4.0 ± 0.3 (4.3)	22	4.5 ± 0.4 (4.3)
DHEAS (μg/dl)	11	138 ± 21 (140)	23	138 ± 18 (121)
E ₂ (pg/ml)	11	65 ± 10 (54)	22	62 ± 6 (53)
P (pg/ml)	4	416 ± 42 (395)	9	519 ± 88 (490)

Data are presented as mean ± SEM (median). Differences were assessed with Wilcoxon rank sum tests. Conversion from conventional to SI units: total T × 3.47 (nmol/liter); insulin × 7.18 (pmol/liter); DHEAS × 27.21 (nmol/liter).

^a $P < 0.05$; ^b $P \leq 0.01$; ^c $P \leq 0.001$; ^d $P \leq 0.0001$, obese *vs.* normal weight controls; *asterisk* denotes the absence of statistical significance after imposing the Bonferroni adjustment for multiple comparisons.

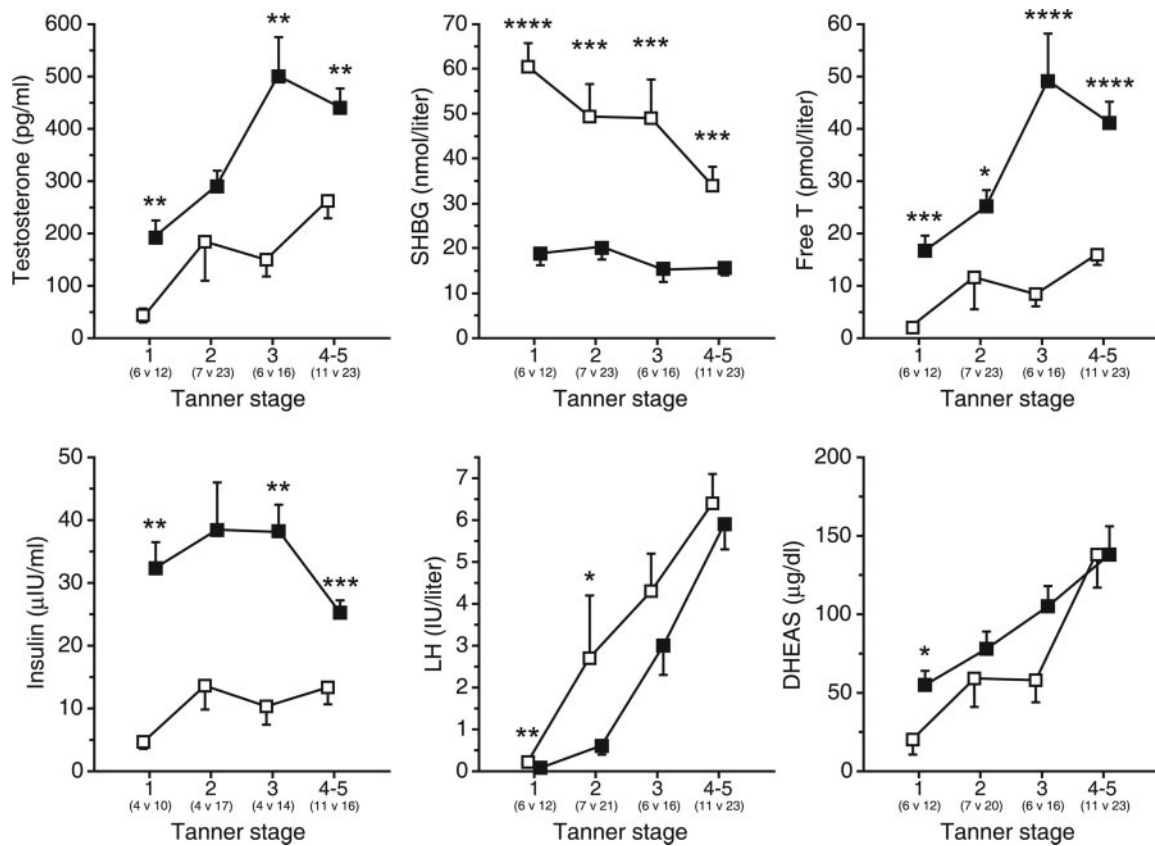


FIG. 1. Graphical representation of observed differences between obese and normal-weight girls grouped by Tanner stage. Data are presented as mean ± SEM. Differences were assessed with Wilcoxon rank sum tests and are also denoted in Table 1. *, $P < 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$ before Bonferroni correction. Conversion from conventional to SI units: total T $\times 3.47$ (nanomoles per liter); insulin $\times 7.18$ (picomoles per liter); DHEAS $\times 27.21$ (nanomoles per liter).

Mean fasting insulin was elevated in obese girls across pubertal development, especially among the pre- and early pubertal (stages 1–3) obese girls, whose mean insulin was 2.8- to 7-fold (median 2.4- to 6.4-fold) higher than their normal-weight counterparts ($P < 0.005$ for Tanner 1 and 3; $P = 0.055$ for Tanner 2). Similarly, compared with their normal-weight counterparts, mean HOMA was higher, being increased 7-fold in Tanner 1 ($P < 0.005$), 2.5-fold in Tanner 2 ($P = 0.08$), 3.7-fold in Tanner 3 ($P < 0.005$), and 1.7-fold in Tanner 4–5 ($P < 0.005$) obese girls.

Mean LH concentrations were lower in obese Tanner stage 1 and 2 girls ($P < 0.05$) but not in girls of later Tanner stages. Mean DHEAS was elevated in Tanner 1 obese girls ($P < 0.05$), but differences were not statistically significant in the Tanner 2 and 3 girls.

Due to blood volume constraints, fewer data are available for E_2 , P, and FSH. Nonetheless, no clear differences of E_2 or P were seen between obese and normal-weight girls in any Tanner stage grouping (Table 1). However, pubertal P concentrations tended to be higher than prepubertal values ($P = NS$). Mean FSH was lower in Tanner 1 obese girls only ($P < 0.05$).

Overnight changes in sex steroids in normal-weight and obese pre- and early pubertal girls

Twenty-three Tanner stage 1–3 girls had sex steroid measurements in the late evening (2200–2300 h) and subsequent

morning (circa 0500–0700 h) (Fig. 3). In the group of normal weight girls ($n = 17$), mean P demonstrated a 2.3-fold overnight increase ($P < 0.001$), whereas mean total T showed a 2.4-fold overnight increase ($P = 0.001$). There was no difference in E_2 .

Mean P and T in the evening were 2.8- and 5.3-fold higher in the group of obese Tanner 1–3 girls ($n = 6$), compared with normal-weight girls ($P < 0.05$ for P; $P = NS$ for T). Mean P and T tended to rise overnight in obese girls (1.4-fold and 1.6-fold increase, respectively), but these changes were not statistically significant.

Cortisol values increased overnight in both normal-weight girls (1.9 ± 0.4 to $12.0 \pm 0.8 \mu\text{g/dl}$) and obese girls (3.1 ± 1.0 to $13.8 \pm 1.8 \mu\text{g/dl}$; $P < 0.05$ for both comparisons). Cortisol values at corresponding time points were not different between normal-weight and obese girls.

Discussion

In our cohort of girls spanning the range of pubertal development, obesity was associated with marked HA and hyperinsulinemia, and this was especially evident in pre- and early pubertal girls. Total T was elevated in obese peripubertal girls: thus, whereas reduced SHBG contributed to the elevation of free T in these subjects, T secretion was also increased. Additionally, DHEAS was elevated in prepubertal obese girls, which is consistent with earlier reports (6, 16). These data augment our earlier observations (7) and provide

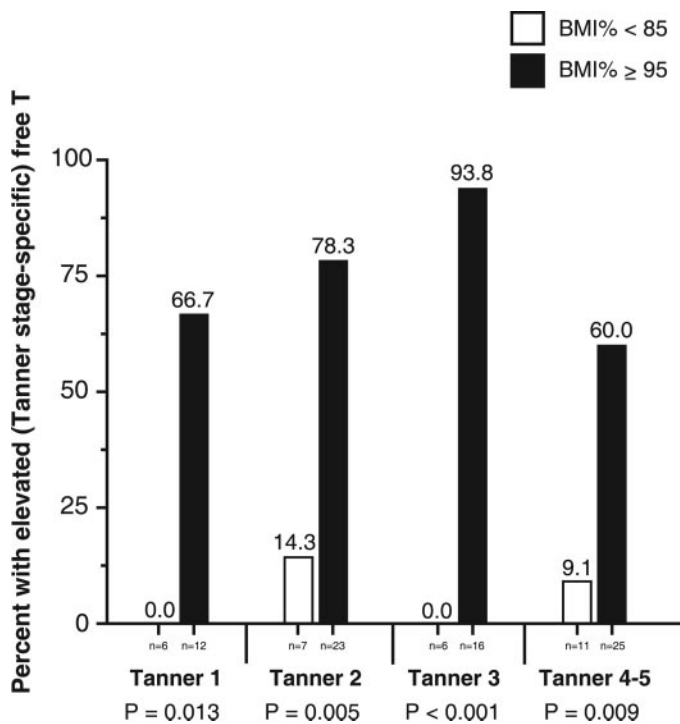


FIG. 2. Proportion of normal-weight and obese girls with HA. The normal range for free T was established for each Tanner stage grouping as the range observed in normal-weight girls with no evidence of hirsutism (and no irregular periods in girls at least 2 yr after menarche). Fisher's exact test was used to compare the proportions with elevated free T within each Tanner stage grouping.

detailed assessment of hormonal values across pubertal maturation. They also emphasize that obesity is commonly associated with HA, with 66–94% of obese Tanner 1–3 girls having an elevated free T.

In keeping with the known association between childhood obesity and both insulin resistance and hyperinsulinemia (17), fasting insulin was elevated in obese peripubertal girls, especially in Tanner 1–3 girls. This finding was supported by elevations in HOMA in obese girls, although it is unclear why both parameters appear disproportionately elevated before and during early puberty. The concomitant elevation of insulin and T in this and other studies (2, 7) suggest a possible mechanism for HA in obese girls; namely, insulin may act as a gonadotropin on the pubertal ovarian theca cell compartment to promote androgen production (1, 18). Insulin also decreases hepatic SHBG production, contributing to elevations in free T (1). Additionally, extensive data support an important role of hyperinsulinemia in the pathogenesis of adult polycystic ovary syndrome (PCOS), primarily via mechanisms discussed above. However, partial correlation analysis in our earlier report (7) revealed a strong association between obesity and free T, even after adjusting for differences in fasting insulin, suggesting that insulin may not be the only mediator of HA in obese girls. We recognize that our assessment of insulin resistance and hyperinsulinemia is limited because insulin values were not available for all subjects, and a single fasting insulin (or HOMA) is an imprecise marker of hyperinsulinemia. Regardless, available data sup-

port a role of insulin in the HA associated with peripubertal obesity.

Our results suggest that early morning LH is relatively low in pre- and early pubertal obese girls but normalizes as pubertal development progresses. This observation in Tanner 1–3 girls is reminiscent of an inverse relationship between BMI and both mean LH and LH amplitude in adults with PCOS (19, 20). However, the association of obesity with advanced physical exam characteristics of puberty has been well described (21). Thus, the lower LH in the Tanner 2 obese girls may in part reflect their younger chronological age and relatively immature GnRH-gonadotrope axis, compared with their normal-weight counterparts. Taken together, the patterns of T, LH, and insulin changes across puberty suggest that insulin plays a particularly important role in obesity-associated HA in pre- and early pubertal girls, with progressive maturation of the hypothalamic-pituitary unit (concomitant with Tanner stage 3) and associated rise in gonadotropins further promoting ovarian T production. Indeed, our earlier partial correlation analysis (7) disclosed a positive correlation between LH and free T after adjusting for differences in age, pubertal stage, BMI, DHEAS, and insulin.

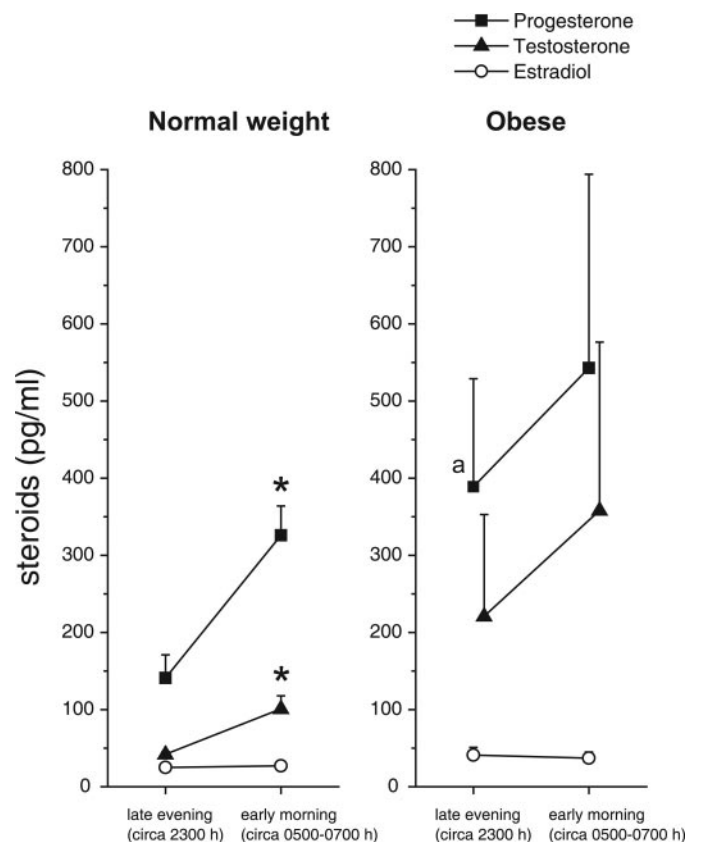


FIG. 3. Overnight changes in sex steroid concentrations (Tanner 1–3). Data are presented as mean \pm SEM. Differences between time points were assessed via Wilcoxon sign rank tests: *, $P \leq 0.001$ (concentrations at \sim 0600 vs. 2300 h). Differences between normal-weight (left panel; $n = 17$) and obese girls (right panel; $n = 6$) were assessed via Wilcoxon rank sum tests: a, $P < 0.05$ (normal weight vs. obese at same time point). Conversion from conventional to SI units: $P \times 3.18$ (picomoles per liter); total T $\times 3.47$ (picomoles per liter); $E_2 \times 3.671$ (picomoles per liter).

We recognize that early-morning LH values do not fully characterize LH secretion during puberty, and further study is required to assess LH secretory patterns in obese peripubertal girls. Overall, however, these data suggest that hyperinsulinemia plays a major role in obesity-associated HA in pre- and early puberty, with both LH and insulin contributing to HA as puberty progresses.

Our group of subjects included a number of girls with clinical evidence of hyperandrogenism. We previously argued (7) that systematic exclusion of such girls may inappropriately lessen the apparent relationship between adiposity and androgen excess. Regardless, analysis after exclusion of such girls was not materially different (data not shown) and did not alter interpretation. Any recruitment bias was especially unlikely to have influenced results in the Tanner 1–3 group: very few of these girls had clinical hyperandrogenism because the manifestations of androgen excess develop slowly.

Previous reports described diurnal variation in T (9, 10) and E₂ (8–11), with peaks occurring between 0600 and 1000 h. P was not measured in these studies, and our results showed that in normal-weight girls, mean P concentrations rose 2.3-fold from 2300 to 0600 h, similar to the increase observed for T. Although P and T appear to increase overnight in obese Tanner 1–3 girls, these differences did not quite reach statistical significance, possibly reflecting the higher evening levels and the limited numbers of obese subjects studied. Overnight changes of E₂ were not observed in our study; this may reflect the timing of surveillance because E₂ tends to peak later in the morning (8, 10, 11).

The origin of pubertal P and T secretion, and overnight increases in plasma concentrations, remains unclear: ovarian P secretion may be stimulated by the overnight increases in LH observed in pubertal girls (22–24), and adrenal P secretion may also increase under ACTH control. The importance of overnight increases of sex steroids during puberty is also unclear but may influence LH pulsatility in pubertal girls. For instance, acute E₂ infusion mitigates the overnight increase of LH secretion in pubertal girls (23). In addition, whereas normal Tanner 1–3 girls demonstrate nocturnal increases in LH (and by inference GnRH) pulse frequency, age-matched girls with gonadal dysgenesis (*i.e.* absent ovarian steroid secretion) do not (22). Of parallel interest is that, when expressed in mass or molar terms, P concentrations exceeded those of both T and E₂ in normal-weight girls, and P is the major regulator of GnRH pulse frequency in adult women (25). Taken together, these findings highlight the importance of delineating the potential regulatory role of P and E₂ in directing diurnal changes of GnRH and LH secretion during female puberty.

In adults with PCOS, relative resistance of the GnRH pulse generator to negative feedback by P plays a role in the persistently rapid GnRH pulse frequency, elevated LH concentrations, and relative FSH deficiency characteristic of this syndrome (26–28). Some adolescents with HA demonstrate a similar feedback defect (29). In adult PCOS, this relative insensitivity is reversed by androgen-receptor blockade (flutamide), suggesting that it is a consequence of HA *per se* (30). We (31) proposed a hypothetical paradigm in which early morning increases in sex steroids (especially P), either di-

rectly by actions on the GnRH pulse generator or via facilitation of higher central nervous system mechanisms, contribute to the reduction of GnRH and LH pulsatility during the following day. Diurnal slowing of GnRH pulsatility would favor FSH synthesis/secretion and subsequent follicular development. In girls with HA-induced decreases in hypothalamic sensitivity to feedback inhibition, overnight increases of sex steroids would not slow GnRH pulsatility during the subsequent day. A persistently (24 h) rapid GnRH pulse stimulus would enhance LH and ovarian androgen secretion and lead to relative FSH deficiency with impaired follicular development. We seek to explore the viability of this paradigm in ongoing and future studies.

In conclusion, peripubertal obesity is associated with hyperinsulinemia and HA, which is particularly marked in pre- and early pubertal girls. Additionally, P and T concentrations in normal Tanner 1–3 girls increase from 2300 to 0600 h. Elucidation of the origin and consequences of the normal overnight increase in sex steroids and obesity-associated HA requires additional study.

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