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The Association of Obesity and Hyperandrogenemia during the Pubertal Transition in Girls: Obesity as a Potential Factor in the Genesis of Postpubertal Hyperandrogenism

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Context: Adolescent hyperandrogenemia is considered a forerunner of adult polycystic ovary syndrome, but its etiology remains uncertain.

Objective: Our objective was to explore the hypothesis that peripubertal obesity is associated with hyperandrogenemia.

Design and Setting: We performed a cross-sectional analysis of data obtained at General Clinical Research Centers.

Subjects: Subjects were 41 obese [body mass index (BMI) for age, ≥95%] and 35 normal-weight (BMI for age, <95%) peripubertal girls.

Intervention: We used pooled blood samples (~0500–0700 h; n = 64) while fasting or single morning (fasting) samples (n = 12).

Main Outcome Measures: We assessed adiposity and androgen concentrations.

Results: BMI correlated with total testosterone (T) ($r_s = 0.59$), SHBG ($r_s = -0.69$), and free T ($r_s = 0.69$); free T was three times as great in obese girls compared with normal-weight girls ($P < 0.0001$ for all). BMI correlated with insulin ($r_s = 0.52$); both insulin and LH correlated with free T ($r_s = 0.45$ and 0.44, respectively; $P < 0.001$ for all). When analyzing early pubertal girls (pubertal stages 1–3; n = 36) alone, BMI correlated with total T ($r_s = 0.65$), SHBG ($r_s = -0.74$), and free T ($r_s = 0.75$); free T was five times as great in obese early-pubertal girls ($P < 0.001$ for all). BMI correlated with insulin ($r_s = 0.65$), and insulin correlated with free T ($r_s = 0.63$, $P < 0.01$ for both). BMI correlated with free T while simultaneously adjusting for age, pubertal stage, insulin, LH, and dehydroepiandrosterone sulfate.

Conclusion: Peripubertal obesity is associated with marked hyperandrogenemia, which is especially pronounced in early puberty. (***J Clin Endocrinol Metab* 91: 1714–1722, 2006**)

THE POLYCYSTIC OVARY syndrome (PCOS) affects approximately 7% of reproductive-aged women and thus may be the most prevalent endocrinological disorder in women (1). The hallmarks of PCOS are hyperandrogenism and ovulatory dysfunction, but PCOS is also associated with obesity and the insulin resistance syndrome (2–4). PCOS likely has a pre- or peripubertal origin in many women, because clinical manifestations frequently have a peripubertal onset. The pathophysiology of PCOS is complex, and its etiology remains unclear.

Both adult women with PCOS (5) and adolescents with hyperandrogenemia (HA) (6–10) demonstrate altered neuroendocrine function with a persistently rapid LH (and by inference GnRH) pulse frequency and elevated serum LH concentrations, which contribute to HA and ovulatory dysfunction. The persistently rapid GnRH pulse frequency characteristic of adult PCOS is partly related to relative GnRH pulse generator resistance to negative feedback by estradiol and progesterone (11, 12), and recent data suggest that a similar abnormality is present in some adolescents with HA (13), a condition thought to precede adult PCOS. This relative hypothalamic insensitivity to negative feedback is likely a result of HA, because it can be reversed in adult PCOS by the androgen-receptor antagonist flutamide (14). This suggests that HA may precede abnormal GnRH pulsatility in adolescent PCOS. However, the putative origin(s) of peripubertal HA remains unknown.

Hyperinsulinemia enhances androgen production in adult women with and without PCOS (2, 3), augmenting ovarian androgen production via actions as a co-gonadotropin with LH (2, 15). Hyperinsulinemia is also associated with decreased SHBG, which further increases free testosterone (T) concentrations (2). Additionally, treatments that reduce hyperinsulinemia generally ameliorate HA and ovulatory dysfunction in both adult and adolescent PCOS (2, 3, 16, 17). Thus, peripubertal obesity with accompanying hyperinsulinemia represents an attractive etiological candidate for peripubertal HA.

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Abbreviations: BMI, Body mass index; CV, coefficient of variation; DHEAS, dehydroepiandrosterone sulfate; HA, hyperandrogenemia; PCOS, polycystic ovary syndrome; T, testosterone.

Previous studies have suggested an association between adiposity and elevated androgens in late pubertal girls (18–21). However, there are few data in early puberty, a critical time when the complex interactions among the hypothalamus, pituitary, and ovary are being developed. One recent study found an association between body mass index (BMI) and both T and dehydroepiandrosterone sulfate (DHEAS) in peripubertal girls (22). Confirmatory data are unavailable, and it remains unclear what mechanisms underlie this relationship in peripubertal girls. Herein we report salient findings in a large group of adolescent girls, including a subset of early pubertal girls.

Subjects and Methods

Subjects

Seventy-six peripubertal girls, ages 7–17 yr, were studied at the University of Virginia Health System (n = 51), the University of Michigan (n = 15), and the University of California at San Diego (n = 10). These volunteers were recruited through advertisements (newspaper or television) or from endocrinology clinics.

Each girl was classified as being either normal weight (gender-specific BMI for age, <95th percentile; n = 35) or obese (BMI for age, ≥95th percentile; n = 41). This definition of pediatric obesity is endorsed by the Centers for Disease Control and Prevention (CDC), the American Academy of Pediatrics (23), the North American Association for the Study of Obesity, and the American Obesity Association. We calculated exact BMI-for-age percentiles by employing a SAS program available from the CDC website (http://www.cdc.gov/nccdphp/dnpa/growthcharts/sas.htm). This program incorporates normative data from the National Health Examination and National Health and Nutrition Examination Surveys (24), which were used to construct the 2000 CDC BMI-for-age growth charts. Individuals were not included or excluded from study based on the presence of absence of hyperandrogenism, hirsutism, or irregular menses.

Each volunteer underwent a detailed history and physical exam including determination of pubertal stage using the Tanner scale for breast development (25). Both palpation and observation of breasts were employed by a single examiner at each time point. (To avoid potential bias related to assessment of androgen-responsive tissues, scales for hair growth were not used.) The normal-weight group included 18 Tanner 1–3 girls (of whom five were Tanner 1) and 17 Tanner 4–5 girls. The obese group included 18 Tanner 1–3 girls (of whom three were Tanner 1) and 23 Tanner 4–5 girls. Clinical diagnoses included normal-weight girls [total n = 30, including idiopathic short stature (n = 4) and idiopathic delayed adolescence (n = 2)], normal weight with hirsutism (n = 1), normal weight with irregular menses (n = 2), normal weight with both hirsutism and irregular menses (n = 2), isolated idiopathic obesity (n = 19), obesity with hirsutism (n = 7), obesity with irregular menses (n = 3), and obesity with both hirsutism and menstrual irregularity (n = 12). Subjects were considered to have irregular menses if they were at least 2 yr post menarche and had an average intermenstrual length of more than 45 d.

Study procedures

We evaluated data from four separate studies involving blood sampling early in the morning after at least 6 h of fasting. Each study was approved by the respective Institutional Review Boards. 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 at least 90 d before study.

Sixty-four subjects underwent frequent blood sampling via an iv catheter in the General Clinical Research Center. Blood samples for later gonadotropin and T determinations were pooled from samples drawn every 10 min for 2 h (~0500–0700 h; n = 49) or every 15 min from 0500–0745 h (n = 15). Insulin, SHBG, and DHEAS were also measured once early in the morning after fasting for at least 6 h. Twelve subjects had a single morning (fasting) blood sample (obtained between 0800 and 1000 h) for the above hormonal measurements. Fasting for at least 6 h was not documented in 15 subjects; therefore, insulin was not measured in these subjects.

Hormonal measurements

All assays were performed at the Center for Research in Reproduction's Ligand Core Laboratory at the University of Virginia Health System. All samples from an individual were analyzed in duplicate in the same assay for each hormone. Samples with measured values below assay sensitivity were assigned the value of the assay's sensitivity.

LH and FSH were measured by chemiluminescence (Diagnostic Products Corp., Los Angeles, CA) [sensitivities, 0.1 and 0.05 IU/liter; intraassay coefficients of variation (CV), 3.2–4.1 and 2.2–2.3%; and interassay CV, 5.3–6.6 and 5.9–6.3%, respectively]. Total T was measured by RIA (Diagnostic Products) (sensitivity, 5.0 ng/dl; intraassay CV, 4.4–10%; and interassay CV, 10.6–12.4%). SHBG and DHEAS were measured by chemiluminescence (Diagnostic Products) (sensitivities, 0.2 nmol/liter and 0.7 μg/dl; intraassay CV, 2.8–3.5 and 5.4–5.6%; and interassay CV, 3.8–5.7 and 6.8–7.2%, respectively). In 35 subjects, insulin was measured by chemiluminescence (Diagnostic Products) (sensitivity, 2.6 μIU/ml; intraassay CV, 3.0–4.0%; and interassay CV, 8.3–8.8%, respectively). In 26 individuals, insulin was measured by RIA (Diagnostics Systems Laboratories, Inc., Webster, TX) (sensitivity, 1.3 μIU/ml; intraassay CV, 7.5–8.5%; and interassay CV, 9.5–21%).

Free T was calculated from total T and SHBG using the following equation (26): $FT = [T - (N)(FT)]/[(K_T)(SHBG) - (K_T)(T) + (N)(K_T)(FT)]$. In this equation, FT is free T (pmol/liter), K_T is association constant of SHBG for T (1.0×10^9 liter/mol), T is total testosterone concentration (ng/dl), SHBG is SHBG concentration (nmol/liter), and N is $(K_A)(C_A) + 1$, where K_A is the association constant of albumin for T (3.6×10^4 liter/mol) and C_A is the concentration of albumin (assumed to be 4.3 g/dl).

Statistical methods

All data are presented as mean ± SEM as well as median (25–75th percentile) unless otherwise noted. We employed nonparametric statistical tests, which are based on ranks of observations and require no assumptions about the underlying distribution of data. (Data transformation did not consistently allow fulfillment of all assumptions for valid parametric testing.) All hypothesis tests were two sided and conducted at the 0.05 level of significance.

Two-sample Wilcoxon tests (*i.e.* Wilcoxon rank sum tests) were used to compare parameters between normal-weight and obese girls. When comparison involved at least 10 observations from each of the two samples, the method of normal approximation was employed; otherwise, exact tests were performed. When making adjustments for multiple comparisons, we employed the highly conservative Bonferroni method. Given nine two-sided, two-sample Wilcoxon comparisons (BMI was not included in this count because BMI should be different given the way groups were defined), the Bonferroni-corrected criterion for significance was 0.0028, obtained by using the formula $\alpha/(2 \times$ number of comparisons).

We explored the association of adiposity (both BMI and gender-specific BMI-for-age percentile) with androgens (total T, free T, and DHEAS), SHBG, insulin, and LH using simple Spearman-rank correlation procedures; resulting estimates of rho are reported as r_s values. We similarly assessed the association of LH and insulin with total T, free T, and SHBG. Partial Spearman-rank correlation procedures were then used to assess simultaneously the relationship between free T (the dependent variable) and the independent variables age, pubertal stage, BMI, LH, and DHEAS. We then performed partial Spearman-rank correlations to assess simultaneously the relationship between free T and the independent variables age, pubertal stage, BMI, LH, DHEAS, and insulin (with insulin data being available for 61 of 76 participants). We also performed the partial correlations using BMI-for-age percentile instead of BMI (and excluding age from the list of partial correlates), but these results are not reported because they were not substantially different from the analyses using BMI. To further assess the possibility that the relationship between BMI and free T is mediated primarily by changes in SHBG, we performed the above partial correlation analyses

using total T as the dependent variable instead of free T. Bonferroni correction was not applied to correlation analyses.

To assess further when putative associations arise, we performed the above analyses for the group of early pubertal girls (pubertal stages 1–3) alone. We also performed the above analyses for the group of premenarcheal girls alone. The results of these two analyses were not substantially different; therefore, only the results for the former analysis are shown. Additionally, to investigate a potential role of recruitment bias in our study (see *Discussion*), we performed all of the above analyses after excluding all adolescents with hirsutism, irregular menses, or both.

Results

Pairwise comparisons (normal weight vs. obese)

When girls of all pubertal stages were considered together (n = 76), the normal-weight group and obese group were of similar age and pubertal stage (Table 1). However, mean total T was 2.1 times as great, SHBG 50% lower, and free T three times as great (median values were two times as great, 58% lower, and 2.9 times as great, respectively) in the obese group compared with normal-weight girls. Mean fasting insulin was 50% (median, 56%) greater in obese girls compared with normal-weight girls, whereas LH and DHEAS concentrations were similar.

When all subjects with hirsutism and/or irregular menses were excluded (Table 2) (remaining n = 49), the mean total T concentration was 1.7 times as great, SHBG 40% lower, and free T two times as great in obese girls (median values, 1.8 times as great, 49% lower, and 2.3 times as great, respectively), despite the fact that obese girls were on average younger and demonstrated lower LH. Tanner stage, fasting insulin, and DHEAS were not significantly different between groups.

When considering only girls earlier in puberty (pubertal stages 1–3; n = 36), mean total T concentration was 2.9 times as great, SHBG 50% lower, and free T five times as great (median values 3.2 times as great, 60% lower, and five times as great, respectively) in the obese group compared with normal-weight girls, even though obese girls were younger on average (Table 1). Fasting insulin concentrations were available for 21 early-pubertal subjects, and the mean value was 2.3 times as great (median, 1.7 times as great) greater in the obese group, a difference that did not achieve statistical significance (unadjusted $P = 0.08$). LH and FSH concentrations were lower in the obese group, although the lower LH did not achieve statistical significance (unadjusted $P = 0.07$). Mean DHEAS was 48% (median, 52%) higher in the obese group.

When early-pubertal subjects with hirsutism were excluded (Table 2) (remaining n = 32), mean total T was 2.2 times as great, SHBG 50% lower, and free T 3.3 times as great (median values, 2.6 times as great, 57% lower, and 4.9 times as great, respectively) in the obese group. The obese group was 2 yr younger on average, and LH and FSH were lower in the obese group. Pubertal stage, insulin, and DHEAS were not demonstrably different.

Further subanalysis of early-pubertal girls revealed that the group of obese pubertal stage 1 girls had a mean free T that was 7.5 times as great (median, 21 times as great) as the group of normal-weight stage 1 girls (mean, 20.9 ± 8.1 *vs.* 2.8 ± 1.4 pmol/liter; median, 22.9 *vs.* 1.1 pmol/liter; total n = 8; $P = 0.07$); the group of obese pubertal stage 2 girls had a mean free T that was 3.7 times as great (median, 4.9 times as great) as the group of normal-weight stage 2 girls (mean, 20.1 ± 3.4 *vs.* 5.5 ± 1.4 pmol/liter; median, 23.1 *vs.* 4.7 pmol/liter; total n = 19; $P = 0.005$); and the group of obese pubertal stage 3 girls had a mean T that was 8.5 times as great (median, 4.3 times as great) as the group of normal-weight stage 3 girls (mean, 84.9 ± 38.8 *vs.* 10.0 ± 1.8 pmol/liter; median, 46.8 *vs.*

TABLE 1. Two-sample Wilcoxon comparisons: comparisons with all subjects included

	All Tanner stages		Tanner 1–3 only	
	Normal weight (n = 35)	Obese (n = 41)	Normal weight (n = 18)	Obese (n = 18)
Age (yr)	12.9 ± 0.4 (13.1, 11.3–14.5)	12.8 ± 0.5 (13.3, 9.8–15.8)	11.2 ± 0.4 (11.6, 9.8–12.9)	9.5 ± 0.4[b,d] (9.1, 8.1–11.1)
Tanner stage	3.3 ± 0.2 (3, 2–5)	3.6 ± 0.2 (4, 2–5)	2.1 ± 0.2 (2, 1–3)	2.0 ± 0.1 (2, 2–2)
BMI (kg/m^2)	20.5 ± 0.7 (20.5, 17.0–24.1)	35.9 ± 1.0 (35.3, 31.6–38.8)	17.5 ± 0.6 (17.0, 15.4–19.0)	33.6 ± 1.4 (33.3, 30.2–36.8)
Total T (ng/ml)	0.19 ± 0.02 (0.20, 0.1–0.24)	0.40 ± 0.04[c] (0.40, 0.23–0.52)	0.12 ± 0.02 (0.10, 0.05–0.21)	0.35 ± 0.07[b] (0.32, 0.09–0.44)
SHBG (nmol/liter)	38.1 ± 3.2 (38.8, 21.1–51.0)	18.9 ± 1.6[c] (16.4, 12.3–24.5)	49.5 ± 4.0 (49.7, 41.3–57.4)	21.6 ± 2.6[c] (19.8, 13.5–25.8)
Free T (pmol/liter)	12.3 ± 1.7 (10.7, 4.7–15.1)	36.4 ± 4.3[c] (30.7, 20.9–45.4)	6.2 ± 1.1 (4.7, 2.5–10.8)	31.0 ± 8.4[c] (23.4, 8.1–33.8)
Fasting insulin (μIU/ml)	16.0 ± 1.8 (14.0, 9.1–23.0) n = 29	25.0 ± 2.5[b,d] (21.9, 14.9–31.5) n = 32	11.1 ± 1.9 (9.7, 6.1–15.7) n = 12	26.0 ± 6.9 (16.2, 12.4–28.6) n = 9
LH (IU/liter)	3.8 ± 0.5 (3.8, 1.2–5.4)	3.9 ± 0.5 (3.6, 0.1–5.4)	2.6 ± 0.7 (1.2, 0.1–4.5)	1.2 ± 0.4 (0.1, 0.1–2.2)
FSH (IU/liter)	3.5 ± 0.3 (3.4, 2.4–4.5)	3.4 ± 0.4 (3.3, 1.4–5.1)	3.6 ± 0.5 (3.2, 2.1–4.7)	2.0 ± 0.6[a,d] (0.8, 0.2–3.1)
DHEAS (μg/dl)	114 ± 16 (81, 38–174)	110 ± 12 (89, 58–138)	50 ± 8 (42, 27–70)	74 ± 9[a,d] (64, 45–98)

Data are presented as mean ± SEM (median, 25–75th percentile). For conversion from conventional to SI units: total T (ng/ml) × 3.467 (nmol/liter); insulin (μIU/ml) × 7.175 (pmol/liter); DHEAS (μg/dl) × 27.211 (nmol/liter).

[a] $P < 0.05$; [b] $P < 0.01$; [c] $P \leq 0.0001$, obese *vs.* normal-weight girls.

[d] Absence of statistical significance after imposing the Bonferroni adjustment for multiple comparisons.

TABLE 2. Two-sample Wilcoxon comparisons: comparisons after exclusion of subjects with hirsutism or irregular periods

	All Tanner stages		Tanner 1–3 only	
	Normal weight (n = 30)	Obese (n = 19)	Normal weight (n = 18)	Obese (n = 14)
Age (yr)	12.5 ± 0.4 (12.8, 10.8–13.7)	10.4 ± 0.6[b,e] (9.8, 8.2–12.8)	11.2 ± 0.4 (11.6, 9.8–12.9)	9.2 ± 0.5[b,e] (8.7, 8.1–10.0)
Tanner stage	3.0 ± 0.3 (3, 2–4)	2.6 ± 0.3 (2, 2–4)	2.1 ± 0.2 (2, 1–3)	1.9 ± 0.1 (2, 2–2)
BMI (kg/m^2)	19.5 ± 0.7 (19.2, 16.9–21.8)	33.7 ± 0.9 (34.5, 30.5–36.9)	17.5 ± 0.6 (17.0, 15.4–19.0)	33.1 ± 1.2 (33.1, 30.2–36.8)
Total T (ng/ml)	0.17 ± 0.02 (0.16, 0.09–0.23)	0.29 ± 0.04[a,e] (0.29, 0.09–0.41)	0.12 ± 0.02 (0.10, 0.05–0.21)	0.26 ± 0.04[a,e] (0.26, 0.09–0.41)
SHBG (nmol/liter)	39.9 ± 3.5 (42.6, 25.3–51.4)	24.1 ± 2.5[b] (21.9, 15.7–28.4)	49.5 ± 4.0 (49.7, 41.3–57.4)	23.2 ± 3.1[d] (21.2, 14.3–25.8)
Free T (pmol/liter)	10.8 ± 1.5 (10.0, 4.4–14.6)	22.3 ± 2.9[b] (22.9, 8.1–33.8)	6.2 ± 1.1 (4.7, 2.5–10.8)	20.7 ± 3.6[c] (22.9, 6.0–29.5)
Fasting insulin (μIU/ml)	16.6 ± 2.0 (14.6, 8.8–24.7) n = 24	19.5 ± 2.9 (15.6, 12.8–28.6) n = 12	11.1 ± 1.9 (9.7, 6.1–15.7) n = 12	16.3 ± 3.4 (13.2, 7.8–28.6) n = 7
LH (IU/liter)	3.7 ± 0.5 (3.9, 0.8–5.4)	2.1 ± 0.6[a,e] (0.1, 0.1–4.1)	2.6 ± 0.7 (1.2, 0.1–4.5)	0.9 ± 0.4[a,e] (0.1, 0.1–1.9)
FSH (IU/liter)	3.6 ± 0.3 (3.6, 2.4–4.6)	2.9 ± 0.7 (1.8, 0.2–6.3)	3.6 ± 0.5 (3.2, 2.1–4.7)	2.1 ± 0.8[a,e] (0.3, 0.1–5.3)
DHEAS (μg/dl)	108 ± 19 (65, 34–155)	90 ± 15 (78, 45–103)	50 ± 8 (42, 27–70)	64 ± 7 (57, 45–96)

Data are presented as mean ± SEM (median, 25–75th percentile). For conversion from conventional to SI units: total T (ng/ml) × 3.467 (nmol/liter); insulin (μIU/ml) × 7.175 (pmol/liter); DHEAS (μg/dl) × 27.211 (nmol/liter).

[a] $P < 0.05$; [b] $P < 0.01$; [c] $P \leq 0.001$; [d] $P \leq 0.0001$, obese *vs.* normal-weight girls.

[e] Absence of statistical significance after imposing the Bonferroni adjustment for multiple comparisons.

11.0 pmol/liter; total n = 9; $P = 0.02$). These differences were attributable to both higher total T and lower SHBG in the obese girls.

Given the use of two different insulin assays over the duration of the studies, we compared both BMI and BMI-for-age percentile between the two insulin assay groups using Wilcoxon rank sum tests, and this revealed no significant differences. Thus, obesity status appeared to be balanced across the insulin assay. Similarly, Wilcoxon rank sum tests revealed no differences in insulin values between the two assays.

Simple Spearman-rank correlations

There were strong positive correlations between adiposity (either BMI or BMI-for-age percentile) and both total T and free T, with estimates of rho (r_s) ranging from 0.45–0.69 (Fig. 1 and Table 3). Adiposity was negatively correlated with SHBG (r_s from −0.61 to −0.69) (Fig. 1 and Table 3) but positively correlated with fasting insulin (r_s from 0.39–0.52) (Fig. 2 and Table 3). DHEAS correlated weakly with BMI (Fig. 2 and Table 3) but not BMI-for-age percentile. Both LH and fasting insulin were positively correlated with both total and free T (r_s between 0.43 and 0.48) (Fig. 3). The above associations remained after excluding subjects with hirsutism and/or irregular menses. Similar associations were seen when evaluating pubertal stage 1–3 subjects in isolation, except that there were no demonstrable correlations between insulin and SHBG and between LH and free T (Table 3 and Figs. 1–3). These differences may in part reflect decreased statistical power attending this smaller subgroup.

Partial Spearman-rank correlation models

In the group as a whole (n = 76), BMI was significantly correlated with free T ($r_s = 0.65$; $P < 0.0001$) (see Table 4), even after adjusting for differences in age, pubertal stage, LH, and DHEAS. LH also had an independent association with free T ($r_s = 0.31$; $P < 0.01$). Similar associations remained when also adjusting for fasting insulin (n = 61) (Table 5), although the association between BMI and free T was not statistically significant when also excluding subjects with hirsutism and/or irregular menses ($P = 0.06$).

In the subgroup of pubertal stage 1–3 girls (n = 36), BMI was independently correlated with free T ($r_s = 0.69$) (Table 4) after adjusting for differences in age, pubertal stage, LH, and DHEAS; a similar association remained after also adjusting for fasting insulin ($r_s = 0.54$; n = 21) (Table 5). LH was not independently predictive of free T in the early pubertal group. Comparable results remained after excluding four subjects with hirsutism.

Results of partial correlation analysis using total T as the dependent variable were similar to those using free T. BMI correlated with total T ($r_s = 0.55$ in girls of all Tanner stages; $r_s = 0.59$ in Tanner stage 1–3 girls; $P < 0.001$ for both) after adjusting for differences in age, pubertal stage, LH, and DHEAS; and similar findings remained after exclusion of all girls with hirsutism and/or irregular menses. When insulin was included as a partial correlate, the independent association between BMI and total T was no longer evident in the group of Tanner stage 1–3 girls alone but remained when all Tanner stages were considered.

Discussion

In our cohort of peripubertal subjects, overweight girls (defined as BMI-for-age ≥ 95th percentile) demonstrate hyperandrogenemia compared with normal-weight girls, consistent with the hypothesis that excessive adiposity promotes hyperandrogenemia. Similar associations between adiposity and androgens were previously observed in older adolescent

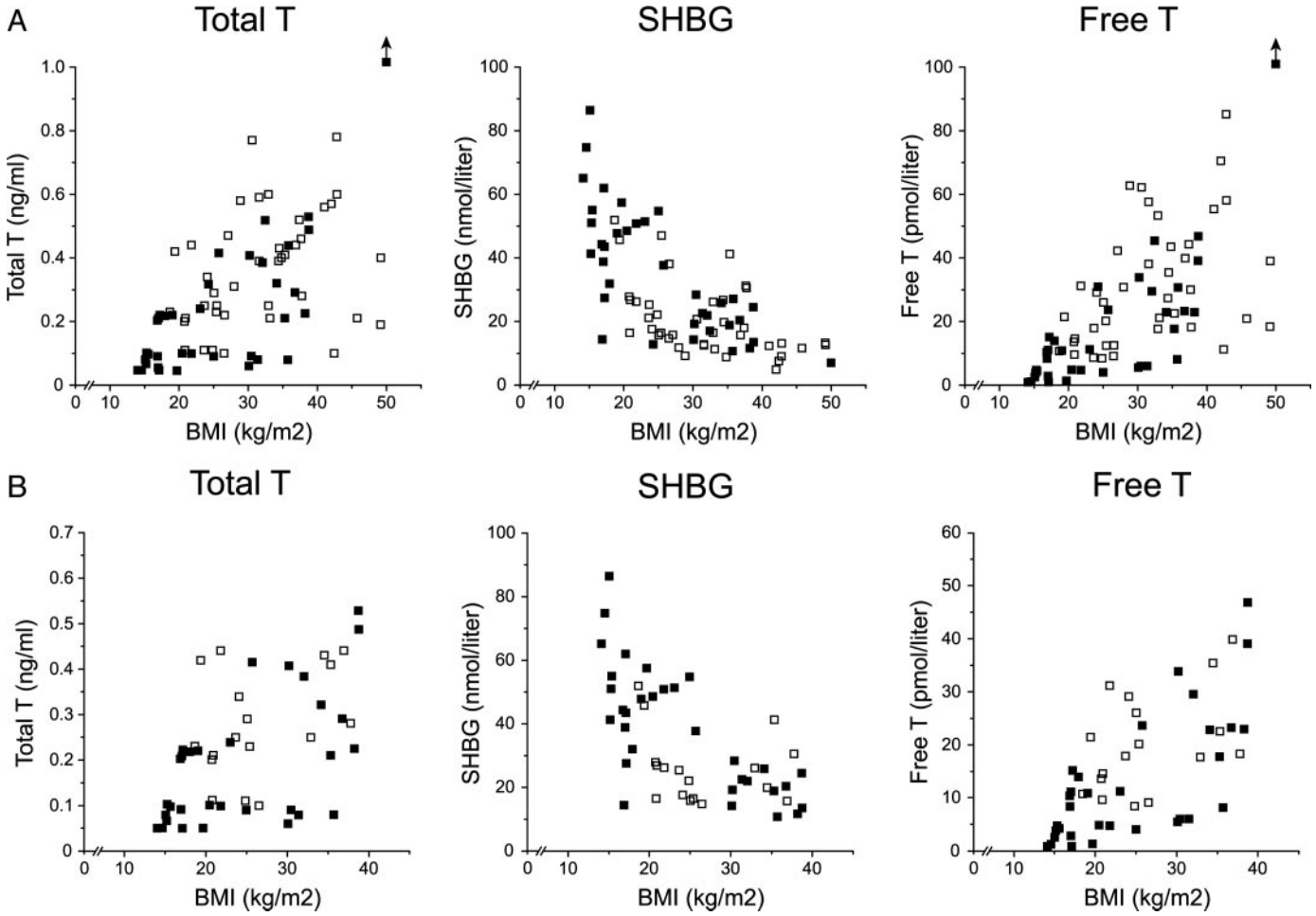

FIG. 1. Relationship between BMI and total T, SHBG, and calculated free T. A, All subjects included; B, subjects without hirsutism and/or irregular periods. ■, Tanner stage 1–3 subjects; □, Tanner stage 4 and 5 girls. Corresponding estimates of rho and *P* values are reported in Table 3. For conversion from conventional to SI units: total T (ng/ml) × 3.467 (nmol/liter).

girls. In overweight (>90th BMI-for-age percentile) late-pubertal (pubertal stages 4–5) Caucasian girls, Wabitsch *et al.* (18) reported increased T and insulin but decreased SHBG, positive correlations between BMI and both free androgen index and insulin, a negative correlation between BMI and SHBG, a positive correlation between insulin and free androgen index, and a negative correlation between insulin and SHBG. In this same study, a 10% weight loss was associated with T and insulin reductions of 10 and 8%, respectively, and an SHBG increase of approximately 50%. In a study of very overweight (BMI-for-age >97th percentile) late-pubertal (pubertal stages 4–5) girls with regular monthly menstrual cycles, central adiposity was positively correlated with total T and glucose-induced insulin changes and negatively correlated with SHBG (20). Another study similarly suggested a positive correlation between BMI and androgens in postmenarcheal adolescent girls (21).

Importantly, hyperandrogenemia was also observed in our subgroup of overweight, early-pubertal (pubertal stages 1–3) girls, and it was relatively more pronounced in this less mature obese group. A small study of prepubertal (aged 7–9 yr) and very-early-pubertal (aged 10–11 yr) girls suggested that total T is approximately 40–50% higher in obese girls compared with age-matched normal-weight girls (27); but these differences were not statistically significant, likely because of the small numbers studied. Another study that included early-pubertal girls suggested an elevated free T index (molar total T divided by molar SHBG) in overweight pubertal girls, a difference largely due to decreased SHBG (19); unfortunately, these groups were not well matched for pubertal stage (*i.e.* there were more late-pubertal girls in the obese group), which potentially confounded these results. A recent study of German girls (22) reported an association between adiposity (BMI) and T. Similar to our results, the median T concentration was four times as great in obese prepubertal girls compared with normal-weight girls, and SHBG was also decreased in obese girls. This study did not evaluate potential etiological factors such as insulin or LH but interestingly suggested that T decreases when obese girls lose weight.

Given the cross-sectional nature of our study, we recognize that a causal relationship (or a directionality of cause and effect) between adiposity and androgens cannot be definitively established on the basis of our data. However, the data are consistent with peripubertal obesity promoting HA. This is also in keeping with other studies demonstrating

TABLE 3. Spearman rank correlations: simple correlations

	All Tanner stages		Tanner 1–3 only	
	(n = 76)	Hirsutism and/or irregular menses excluded (n = 49)	(n = 36)	Hirsutism excluded (n = 32)
BMI *vs.*				
Total T	0.59[d]	0.58[d]	0.65[d]	0.58[c]
SHBG	−0.69[d]	−0.67[d]	−0.74[d]	−0.73[d]
Free T	0.69[d]	0.67[d]	0.75[d]	0.72[d]
Fasting insulin	0.52 (n = 61)[d]	0.52 (n = 36)[b]	0.65 (n = 21)[b]	0.55 (n = 19)[a]
LH	0.14	0.02	−0.01	−0.09
DHEAS	0.25[a]	0.31[a]	0.39[a]	0.34
BMI-for-age percentile *vs.*				
Total T	0.45[d]	0.44[b]	0.52[b]	0.45[b]
SHBG	−0.61[d]	−0.64[d]	−0.75[d]	−0.72[d]
Free T	0.55[d]	0.56[d]	0.65[d]	0.62[c]
Fasting insulin	0.39 (n = 61)[b]	0.33 (n = 36)[a]	0.46 (n = 21)[a]	0.34 (n = 19)
LH	−0.14	−0.20	−0.22	−0.30
DHEAS	0.06	0.13	0.25	0.20
Fasting insulin *vs.*				
Total T	0.43 (n = 61)[c]	0.52 (n = 36)[b]	0.66 (n = 21)[b]	0.54 (n = 19)[a]
SHBG	−0.30 (n = 61)[a]	−0.32 (n = 36)	−0.39 (n = 21)	−0.24 (n = 19)
Free T	0.45 (n = 61)[c]	0.55 (n = 36)[c]	0.63 (n = 21)[b]	0.50 (n = 19)[a]
LH *vs.*				
Total T	0.48[d]	0.46[c]	0.36[a]	0.36[a]
SHBG	−0.21	−0.07	0.10	0.16
Free T	0.44[d]	0.38[b]	0.24	0.21

Estimates of rho are presented.
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.
[d] $P \leq 0.0001$.

reduced androgens with weight loss in adult women (2), prepubertal girls (22), and late-pubertal girls (18).

Partial correlation analyses suggest that obesity is independently associated with elevated free T (*i.e.* positively correlated even after adjusting for differences in age, Tanner stage, LH, insulin, and DHEAS). Although an inverse relationship between adiposity and SHBG contributes to this relationship, the positive correlation (by both simple and partial correlation analyses) between adiposity and total T suggests that this is not the only factor involved.

Our group of subjects included a number of girls with clinical hyperandrogenism. In an effort to assess for potential recruitment bias, we analyzed the data after excluding girls with clinical manifestations that could reflect excess androgens. We have reported both analyses, because *a priori* exclusion of all subjects with symptoms or signs of hyperandrogenism may bias the results in a way that lessens the apparent relationship between adiposity and hyperandrogenemia (*i.e.* in a study of the potential relationship between adiposity and androgens, systematic exclusion of all subjects with external evidence of hyperandrogenemia may not be completely valid). This subgroup analysis was consistent in showing significant associations between adiposity and elevated T. Thus, although recruitment bias may have influenced the results for our larger cohort, it does not appear to account fully for these findings. Similarly, recruitment bias is unlikely to have influenced results in the group of early-pubertal girls, because few (only four of 32) of these participants had signs or symptoms of potential hyperandrogenism. This would be consistent with a temporal requirement for the development of clinical evidence of hyperandrogenemia.

Some of our comparisons revealed a younger average age in the overweight group compared with their normal-weight counterparts. This is consistent with the well-described association of earlier sexual maturation with obesity. For instance, in one large cross-sectional study of NHANES III data, girls with early sexual maturation were twice as likely to be obese than other girls (28). The causal nature of this relationship remains uncertain, but it is possible that increased androgens and enhanced aromatization to estrogens may play a role in this phenomenon.

Numerous studies confirm that childhood obesity is associated with insulin resistance, hyperinsulinemia, and an increased risk of later diabetes (29). For example, a cross-sectional study demonstrated that BMI was negatively correlated with insulin sensitivity index (by frequently sampled iv glucose tolerance test, Bergman modified minimal model) in Caucasian pubertal stage 2 and 3 girls, even when overweight girls (ideal body weight > 120%) were excluded (30). Because there is good evidence that hyperinsulinemia promotes ovarian androgen production in adult women, we hypothesized that a similar mechanism may be responsible for hyperandrogenism in peripubertal girls. This is supported by the strong positive (simple) correlations between adiposity and fasting insulin in our study. The independent association between BMI and free T appeared to lessen when also adjusting for insulin, but we were unable to demonstrate an independent relationship between fasting insulin and free T via partial correlation modeling. We believe this may be explained by several factors. First, a single fasting insulin is not a precise measure of either insulin sensitivity or overall insulinemia. Additionally, two different insulin assays were used over the duration of the study, which may have masked

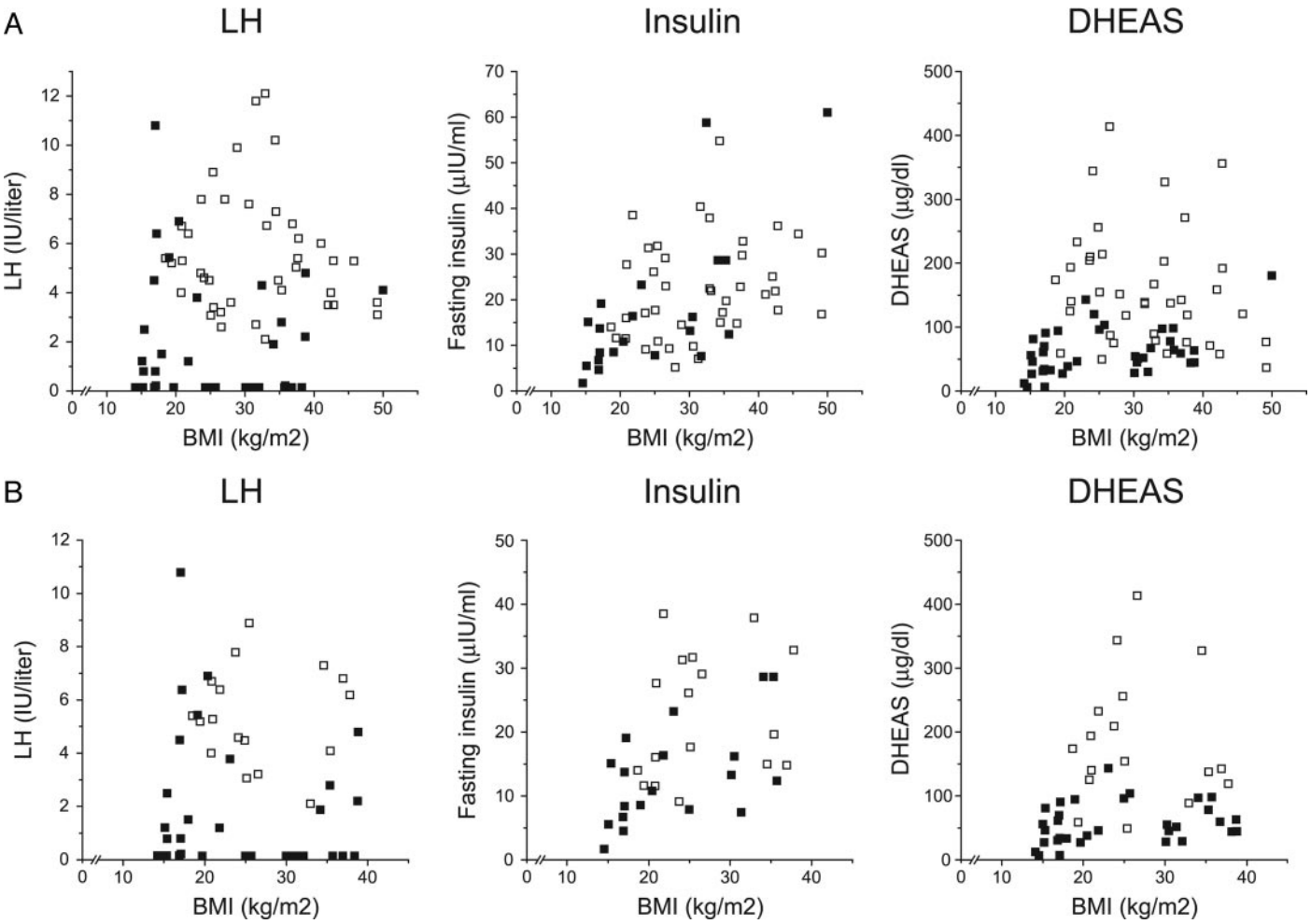

FIG. 2. Relationship between BMI and LH, fasting insulin, and DHEAS. A, All subjects included; B, subjects without hirsutism and/or irregular periods. ■, Tanner stage 1–3 subjects; □, Tanner stage 4 and 5 girls. Corresponding estimates of rho and *P* values are reported in Table 3. Conversion from conventional to SI units: insulin (μIU/ml) × 7.175 (pmol/liter); DHEAS (μg/dl) × 27.211 (nmol/liter).

correlations (importantly, obesity status was determined to be balanced between assay methods). Also, fasting insulin was determined in only 61 of 76 subjects, reducing statistical power.

Elevated DHEAS has been reported in obese pre- and peripubertal girls (22, 31). We also found elevated DHEAS in obese early-pubertal girls, but this difference was not statistically significant after Bonferroni correction. Additionally, the correlation between BMI and DHEAS was not evident when BMI was normalized for age (BMI-for-age percentile), and the relationship between BMI and free T remained after adjusting for multiple variables including DHEAS. Taken together, these findings suggest that, whereas obesity may be associated with increased adrenal androgens in early puberty, abnormal adrenal androgen production does not fully account for the association between adiposity and free T.

Previous studies have demonstrated a negative correlation between BMI and both mean LH and LH amplitude in adults with PCOS (32, 33). We found no simple correlation between BMI and mean LH in our cohort of adolescents. This may reflect the marked heterogeneity of LH concentrations across puberty, with an expected 4- and 9-fold increase of LH pulse frequency and amplitude, respectively, during puberty (34). This may also reflect our assessment of LH over a limited time window, *i.e.* 2 h compared with 8–24 h in the above studies (32, 33). It also remains possible that the relationship between adiposity and LH secretion in peripubertal adolescents is different from that in adults.

LH is the proximate physiological stimulus promoting ovarian androgen production. Partial correlation analysis of our entire cohort suggests that LH is independently correlated (positively) with free T when simultaneously adjusting for differences in age, pubertal stage (*i.e.* Tanner stage), BMI, DHEAS, and insulin. Although this finding is consistent with the possibility that preexisting neuroendocrine regulatory defects promote hyperandrogenemia via relatively excessive LH secretion, it is also consistent with an effect of peripubertal hyperandrogenemia in causing excess LH secretion.

In adult PCOS, relative insensitivity to negative feedback by progesterone is likely mediated by excess androgens, because it is reversed with the androgen-receptor blocker flutamide (14). Some with adolescent HA exhibit a similar hypothalamic insensitivity to progesterone negative feedback (13). These findings suggest the possibility that HA may

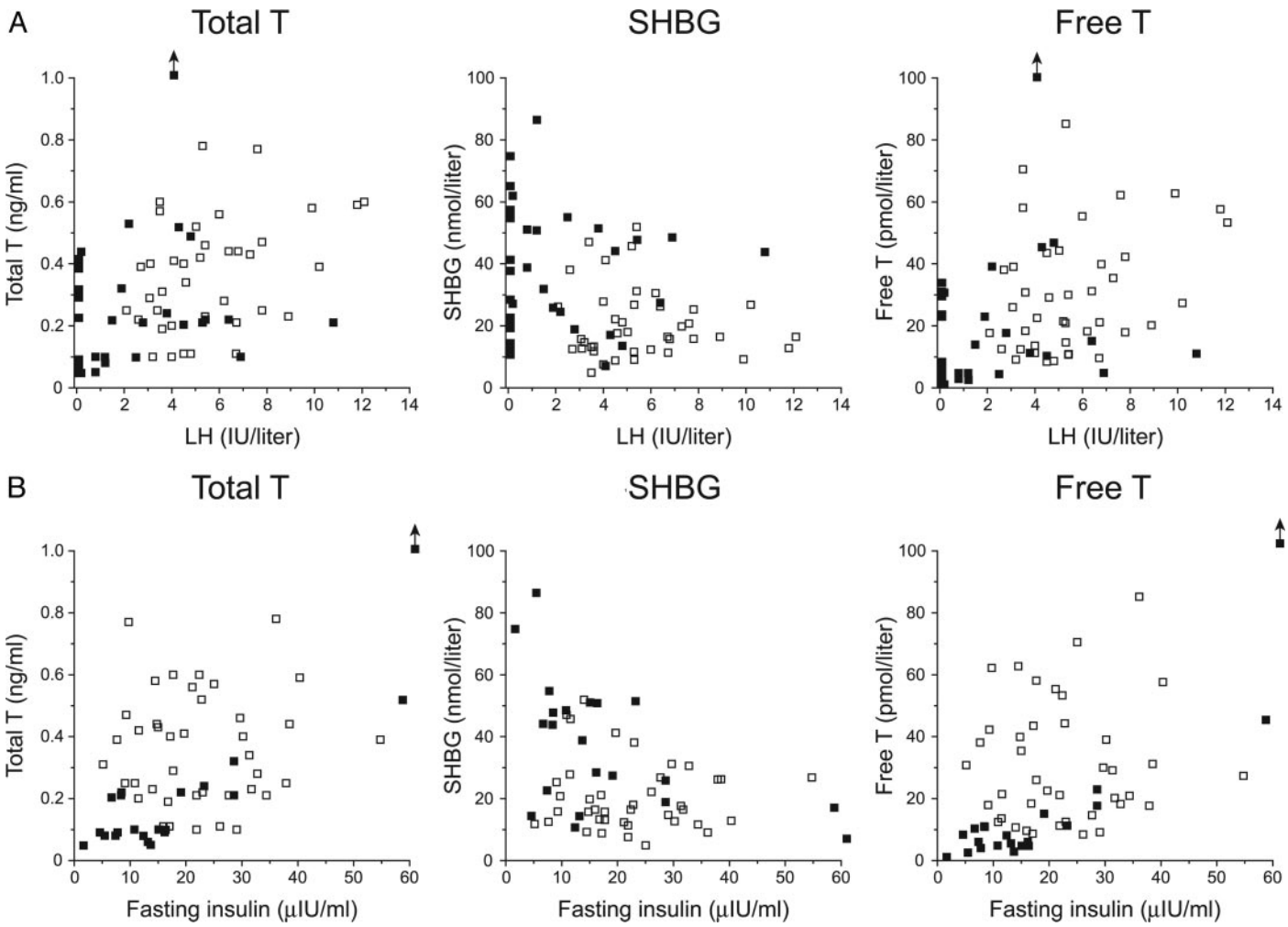

FIG. 3. Relationship of LH (A) and fasting insulin (B) to total T, SHBG, and calculated free T. ■, Tanner stage 1–3 subjects; □, Tanner stage 4 and 5 girls. Corresponding estimates of rho and *P* values are reported in Table 3. Conversion from conventional to SI units: total T (ng/ml) × 3.467 (nmol/liter); insulin (μIU/ml) × 7.175 (pmol/liter).

precede abnormal GnRH pulsatility in adolescent PCOS. We therefore propose that, in some individuals, obesity may play an early role in the genesis of PCOS by contributing to increased androgen production. Hyperandrogenemia may in turn result in neuroendocrine dysfunction in susceptible peripubertal girls, resulting in increased LH and decreased FSH. These abnormalities would then enhance ovarian androgen production and limit follicular development, respectively, thus supporting the progression toward the adult PCOS phenotype.

TABLE 4. Spearman rank correlations: partial Spearman-rank correlation models with dependent variable free T and independent variables (partial correlates) age, pubertal stage, BMI, LH, and DHEAS

	All Tanner stages		Tanner 1–3 only	
	(n = 76)	Hirsutism and/or irregular menses excluded (n = 49)	(n = 36)	Hirsutism excluded (n = 32)
Age	−0.08	−0.14	−0.14	−0.05
Tanner stage	0.06	0.07	0.10	0.03
BMI	0.65[c]	0.64[c]	0.69[c]	0.67[c]
LH	0.31[b]	0.33[a]	0.24	0.24
DHEAS	0.11	0.10	0.27	0.21

Estimates of rho are presented.
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P \leq 0.0001$.

TABLE 5. Spearman rank correlations: partial Spearman-rank correlation models with dependent variable free T and independent variables (partial correlates) age, pubertal stage, BMI, fasting insulin, LH, and DHEAS

	All Tanner stages		Tanner 1–3 only	
	(n = 61)	Hirsutism and/or irregular menses excluded (n = 36)	(n = 21)	Hirsutism excluded (n = 19)
Age	0.05	−0.09	0.37	0.48
Tanner stage	0.14	0.06	−0.30	−0.41
BMI	0.53[c]	0.35	0.54[a]	0.56[a]
Fasting insulin	0.02	0.22	0.05	−0.12
LH	0.40[b]	0.46[b]	0.42	0.37
DHEAS	0.09	0.20	0.31	0.35

Estimates of rho are presented.
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P \leq 0.0001$.

In summary, peripubertal obesity is associated with hyperandrogenemia, a finding that appears to be especially pronounced in early puberty. Insulin and LH contribute to increased T in obese peripubertal adolescents, but other factors associated with obesity may also mediate this association.

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