Prevalence of Hyperandrogenic States in Late Adolescent and Young Women: Epidemiological Survey on Italian High-School Students

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Context: Most of the estimates of the prevalence of hyperandrogenic states refer to the general adult population.

Objective: The objective of the study was to estimate the prevalence of hyperandrogenic states in late adolescence and youth and to evaluate potential independent predictors.

Design: This was a cross-sectional study.

Setting: The study was conducted in high schools.

Patients: Patients included female students, aged 16–19 years.

Main Outcome Measures: The study protocol was designed with 3 possible levels of participation: the first level consisted of a self-compiled questionnaire; the second level added a medical examination; and the third level added a blood sample for laboratory testing. Liquid chromatography-tandem mass spectrometry was used to measure total testosterone, and a reference interval was established in-house.

Results: We offered participation to 2052 students, and 1469 of those compiled the questionnaire. Of these, 1038 were examined, and 519 also provided blood samples. Two hundred three of the 1038 examined students and 125 of the 519 students who provided blood samples were subsequently excluded because of treatment with oral contraceptives or because of endocrine disorders. In the sample of women with a questionnaire + a medical examination, 13% were affected by isolated menstrual irregularity, 16.1% by isolated clinical hyperandrogenism, and 3.8% by both states. A similar prevalence of isolated menstrual irregularity (10.2%) and isolated clinical hyperandrogenism (16.7%) was found in the subsample of women with laboratory tests; in addition, 6.6% showed isolated hyperandrogenemia, and 4.3% proved to be affected by polycystic ovary syndrome.

Conclusions: This study provides for the first time a reliable assessment of the prevalence of hyperandrogenic states in late adolescent and young females and confirms that hyperandrogenic disorders originate at a young age. **(*J Clin Endocrinol Metab* 98: 1641–1650, 2013)**

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Abbreviations: BMI, body mass index; CV, coefficient of variation; HOMA-IR, homeostasis model assessment for insulin resistance; LC-MS/MS, liquid chromatography-tandem mass spectrometry; OR, odds ratio; PCOS, polycystic ovary syndrome; QUICKI, quantitative insulin sensitivity check index.

Polycystic ovary syndrome (PCOS) and the other less frequent hyperandrogenic states usually arise during adolescence and youth. However, most of the estimates of their prevalence refer to the general adult population (1–7), and the few available data on adolescent and young females come from small studies that applied the same diagnostic criteria used for adults (8, 9). Nevertheless, none of these diagnostic criteria could be confidently used in adolescence and youth. In particular, menstrual irregularities and oligoanovulation or enlarged ovaries with a polycystic appearance may reflect normal adolescence dynamics and may overestimate the prevalence of androgen excess disorders in the early postmenarchal years (10). At variance, hirsutism may underestimate the prevalence of androgen excess disorders because hirsutism is less prominent during the adolescent period compared with adulthood because hair growth becomes thick and coarse with increasing duration of androgen exposure (11). Similar behavior is conceivable for androgenic alopecia, although there are no data to support this. Serum androgen levels (in particular, testosterone) probably provide the best measure of androgen excess in adolescence and youth, but a specific reference interval has to be created and a sensitive assay has to be used because there is a physiological increase in androgen levels during normal puberty (12).

To provide an estimate of the prevalence of hyperandrogenic states in adolescence and youth and to evaluate potential independent predictors, we performed a 3-stage, cross-sectional study on a large sample of high school female students. We used liquid chromatography-tandem mass spectrometry (LC-MS/MS) to determine testosterone levels, and we created a specific reference interval. In addition, we included females in late adolescence and youth to avoid the early postmenarchal age and therefore to increase the predictive value of menstrual abnormalities and hirsutism in identifying androgen excess disorders.

Materials and Methods

Study design

High school female students, aged 16–19 years, were recruited in 2 provinces (Bologna and Forlì-Cesena) of the Emilia-Romagna region of Italy from October 2007 to November 2008. Students were recruited from different types of high schools (lyceums, technical, and professional institutes). The study protocol was designed with 3 possible levels of participation characterized by a growing commitment: the first level consisted of a self-compiled questionnaire aimed at collecting some sociodemographic information and at assessing a self-estimate of clinical hyperandrogenism (by descriptive modified Ferriman-Gallwey-mFG scoring sheet and Ludwig scale sheet for hirsutism and alopecia, respectively) and menstrual irregularities; the second level added a medical examination, in which a structured interview and a physical examination were carried out by a trained medical doctor; and participants were finally requested to provide a blood sample for laboratory testing (third level of participation). Each evaluation was performed in the morning during school hours.

The structured interview was carried out to collect data on demographic status, smoking, drug therapy, familial and personal history of diseases, gynecological history, menstrual dating, and irregularity. During the physical examination, hirsutism was scored, the presence or absence of acne, androgenic alopecia, and acanthosis nigricans were recorded, and height, weight, waist and hip circumferences, and blood pressure were measured. A bioimpedenziometric estimate of free fatty mass and fatty mass was also performed with a single-frequency, 50-kHz bioelectrical impedance analyzer (BIA 101 RJL; Akern Bioresearch, Florence, Italy) according to the standard tetrapolar technique, with the subject in supine position and the electrodes placed on the dorsal surface of the right foot and ankle and the right wrist and hand. Body composition was calculated by applying the software provided by the manufacturer, which incorporated validated predictive equations for total body water, free fatty mass, and fatty mass (13, 14). Body mass index (BMI) was computed as the ratio between weight (in kilograms) and height (in square meters). Hirsutism was scored through the mF-G score (15); androgenic alopecia through the Ludwig scale (16); face, back, and chest acne through the Burke-Cunliffe graded score (17); and neck, axilla, knuckles, elbows, and knees acanthosis nigricans through the Burke quantitative scale (18). Dates of menses in the previous year were recorded during the structured interview and used to define menstrual irregularity.

Blood samples were taken in fasting condition between 8:00 and 10:00 AM, regardless of the menstrual phase. However, the last menstrual date was recorded and the cycle day was calculated. All samples were centrifuged immediately and stored at −80°C until assayed.

The study was approved by the institutional review board, and all participants provided written informed consent (in the case of minors, parents signed an appropriate consent form).

Assays

Serum hormones and metabolites [intra- and interassay coefficients of variation (CV)] were measured at the Central Laboratory of S. Orsola-Malpighi Hospital by Modular Analytics E170 (Roche Diagnostics, Mannheim, Germany): glucose (1.1% and 1.9%); triglycerides (<1.5% and 1.8%); total cholesterol (<1.0% and 2.7%); high-density lipoprotein cholesterol (<0.95% and 1.3%); insulin (1.5% and 4.9%); uric acid (0.5% and 0.7%); TSH (3.0% and 7.2%); prolactin (1.7% and 2.0%); LH (1.2% and 2.2%); FSH (2.8% and 4.5%); and estradiol (3.3% and 4.9%). SHBG was measured by Immulite 2000 (Siemens Healthcare Diagnostics, Deerfield, Illinois): intra- and interassay CVs were 4.2% and 6.6%, respectively.

Total testosterone, 17-hydroxyprogesterone, progesterone, and cortisol were measured at the Center for Applied Biomedical Research of St Orsola-Malpighi Hospital by LC-MS/MS: the intra- and interassay CVs were below 8% and 11%, and trueness ranged between 83.7% and 104.9% for all the analytes. The assay description and validation data are reported elsewhere (19). In-house reference intervals (centiles of 2.5–97.5), according to the menstrual phase, were established using a subgroup of 149 healthy, normal-weight, and untreated female students (20).

LH, FSH, estradiol, and progesterone levels were used, in association with the cycle day, to define the phase of the menstrual cycle (follicular, luteal, and peak) of each sample; 298 subjects provided blood samples in the follicular phase, 205 in the luteal phase, and 16 in the hormonal peak. Insulin resistance and sensitivity were estimated using the homeostasis model assessment for insulin resistance (HOMA-IR) (21) and the quantitative insulin sensitivity check index (QUICKI) (22). The free androgen index was calculated (23).

Criteria for the definition of hyperandrogenic states

Menstrual irregularity was considered when a subject had more than 6 cycles with a length of more than 35 days per year or the lack of menstrual bleeding for 3 consecutive months, respectively (24). Clinical hyperandrogenism was defined by the presence of hirsutism, represented by an mF-G score of 8 or more, or the presence of androgenic alopecia (25). Acne was not considered a clinical sign of hyperandrogenism due to the young age of the participants (25). Hyperandrogenemia was defined by a circulating total testosterone level above the 97.5th centile of the reference interval, according to the phase of the menstrual cycle: 0.416 ng/mL for the follicular phase, 0.553 ng/mL for the luteal phase, and 0.529 ng/mL for the peak (20). The combination of menstrual irregularity with clinical hyperandrogenism and/or hyperandrogenemia, after the exclusion of specific etiologies (thyroid dysfunction, hyperprolactinemia, nonclassic 21-hydroxylase deficiency, Cushing's syndrome, androgen secreting tumors, and anabolic drug use and abuse) was used to define PCOS (25).

Statistical analysis

To detect potential differences in the distribution of all recorded variables across groups defined by either level of participation [1) declined participation; 2) questionnaire only; 3) questionnaire + medical examination; or 4) questionnaire + medical examination + blood sample] or clinical status [1) normal; 2) isolated menstrual irregularity; 3) isolated clinical hyperandrogenism: hirsutism and/or androgenic alopecia; or 4) menstrual irregularity and clinical hyperandrogenism], we used the χ^2 test for categorical variables and 1-way ANOVA with Sidak correction for continuous variables.

To test the independent association between all recorded covariates and clinical state, a polytomous logistic model was used (26). Three odds ratios (ORs) were therefore obtained for each predictor variable: using normal women as the reference category, the first OR referred to women with isolated menstrual irregularity, the second to women with isolated clinical hyperandrogenism, and the third to women with both menstrual irregularity and clinical hyperandrogenism.

We also fitted 3 binomial logistic regression models using the above dependent variables separately. In all models, all recorded covariates were included a priori, although several covariates were then excluded because of multicollinearity (ie, mean weight and BMI) and to reduce the likelihood of overfitting (if nonsignificant). Standard postestimation tests were used to assess final model validity [Hosmer-Lemeshow test for evaluation of the goodness of fit, area under the receiver-operating characteristic curve for predictive power (27)], performing influential observation analyses [using standardized residuals, change in Pearson, and deviance χ^2 (28)], and testing for potential interaction and higher-power terms. When analyses were repeated excluding influential observations, the results did not vary significantly; moreover, the percentage of outliers was less than 10% in all models (thus, all observations were included). Notably, because the results of the polytomous and separate binomial logistic models were substantially similar, only the polytomous regression model was shown to avoid redundancy.

In the presence of a relevant selection bias, the relationship between covariates and clinical status might have been different in the subsample of women who compiled the questionnaire and underwent the medical examination (and refused to provide blood samples), as compared with women who also provided blood samples. To investigate this issue, we performed all the above analyses of the potential predictors of hyperandrogenic states 3 times: 1) considering only women who compiled the questionnaire and underwent the medical examination but refused to provide blood samples; 2) including only women who also provided blood samples; and 3) including all women who compiled the questionnaire and underwent the medical examination (providing or not providing blood samples).

As a secondary explorative analysis, we repeated all analyses using a more specific definition of clinical state, which also took T levels into account. Such a definition was available only after laboratory testing, and all analyses were thus restricted to the women who also provided blood samples. Although we again

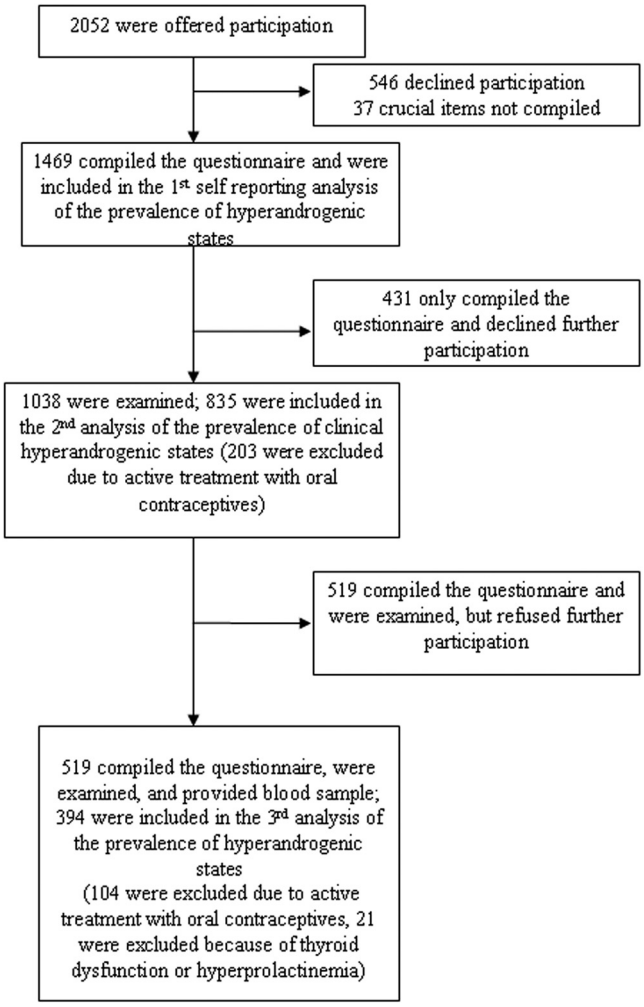

Figure 1. Flow of the study participants.

used polytomous logistic regression, the number of covariates to be included in the model was higher because new variables were available (ie, cholesterol level). Thus, given the high potential for overfitting in some comparisons (ie, to evaluate the predictors of the isolated increase in testosterone levels), for which only a small number of successes was available (n = 26), we could not include all the recorded covariates a priori and included only those significant after a stepwise forward process. In any case, the potential for the overfitting of this analysis remained high, and the results should be interpreted with caution.

Statistical significance was defined as a 2-sided $P < .05$ for all analyses, which were performed using STATA 10.1 (Stata Corp, College Station, Texas, 2007).

Role of the funding source

The funding source had no involvement in the collection, analysis, and interpretation of data.

Results

Participants

We offered participation to 2052 high school female students. As shown in Figure 1, 1469 compiled the questionnaire (71.6%). Of these, 1038 (71.0%) were also examined, and 519 (35.3%) also provided blood samples for laboratory testing. Accordingly, 3 separate groups of students were identified based upon the level of participation: 1) questionnaire only (n = 431); 2) questionnaire + medical examination (n = 519); and 3) questionnaire + medical examination + blood sample (n = 519). The 3 groups showed a similar age distribution, but students from lyceums were more likely to participate in the advanced stages of the study

Table 1. Prevalence of Hyperandrogenic State Overall and by Level of Participation

Variables, % (n)	Questionnaire Only	Questionnaire + Medical Examination	Questionnaire + Medical Examination + Blood Sample	*P* Value[a]	Overall Sample
Clinical state based on questionnaire	(n = 431)	(n = 519)	(n = 519)		(n = 1469)
Normal	74.9	67.2	71.7	>.05	71.0
Isolated menstrual irregularity	10.4	14.6	11.9	>.05	12.5
Isolated clinical hyperandrogenism	11.4	14.5	13.7	>.05	13.3
Menstrual irregularity and clinical hyperandrogenism	3.3	3.7	2.7	>.05	3.2
Mean age, y (SD)					
At the time of the study start	17.6 (0.9)	17.5 (1.0)	17.5 (0.9)	>.05	17.5 (1.0)
At menarche	12.3 (1.3)	12.3 (1.2)	12.2 (1.3)	>.05	12.2 (1.3)
Clinical state based on questionnaire and medical examination	(n = 0)	(n = 420)[b]	(n = 415)[b]		(n = 835)[b]
Normal		66.4	67.7	>.05	67.1
Isolated menstrual irregularity		14.3	11.8	>.05	13.0
Isolated clinical hyperandrogenism		14.3	17.8	>.05	16.1
Menstrual irregularity and clinical hyperandrogenism		5.0	2.7	>.05	3.8
Mean age, y (SD)					
At the time of the study start		17.4 (1.0)	17.4 (0.9)	>.05	17.4 (1.0)
At menarche		12.3 (1.2)	12.2 (1.3)	>.05	12.3 (1.3)
Clinical state based on questionnaire and medical examination and blood sample	(n = 0)	(n = 0)	(n = 394)[c]		(n = 394)[c]
Normal			60.9		60.9
Isolated menstrual irregularity			10.2		10.2
Isolated clinical hyperandrogenism			16.7		16.7
Isolated hyperandrogenemia			6.6		6.6
Clinical hyperandrogenism and hyperandrogenemia			1.3		1.3
PCOS[d]			4.3		4.3
Mean age, y (SD)					
At the time of the study start			17.4 (0.9)		17.4 (0.9)
At menarche			12.2 (1.3)		12.2 (1.3)

[a] χ^2 test for categorical variables.

[b] Two hundred three oral contraceptive users were excluded.

[c] One hundred four oral contraceptive users were excluded; 21 subjects were excluded because of thyroid dysfunction or hyperprolactinemia.

[d] PCOS defined by the combination of menstrual irregularity with clinical hyperandrogenism and/or hyperandrogenemia, after the exclusion of specific etiologies.

than those from professional institutes: most of the students who declined participation came from professional institutes (47.5%), whereas most of the students who participated in all phases of the study came from lyceums (57.6%) (Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

Reliability of the self-compiled questionnaire in identifying hyperandrogenic states

We compared the diagnosis of each student after the questionnaire and after the medical examination (used as a gold standard) in the group of subjects who performed both evaluations to quantify the reliability of self-reporting in defining the correct clinical status (Supplemental Table 2). Overall, the level of agreement was quite high (74.5%), but the reliability of the questionnaire largely varied, depending on the diagnosis. In particular, the specificity of self-reporting was relatively high (83.6% of subjects who were classified as normal based on the questionnaire were defined as normal after the medical examination). Moreover, the questionnaire showed a high reliability (sensitivity) also in students diagnosed with isolated menstrual irregularity by questionnaire (diagnosis was confirmed after the examination in 78.8% of the subjects). In contrast, only 33.6% and 39.3% of subjects diagnosed, based on the questionnaire, as having isolated clinical hyperandrogenism or both menstrual irregularity and clinical hyperandrogenism, respectively, had their diagnosis confirmed after the examination. In particular, after the examination, most cases of isolated clinical hyperandrogenism were classified as normal (66.4%), and most cases of combined menstrual irregularity and clinical hyperandrogenism were diagnosed as menstrual irregularity only (53.6%).

Prevalence of each hyperandrogenic status

Overall, 835 students were included in the analysis of the prevalence of hyperandrogenic states based on medical examination (203 were excluded due to active treatment with oral contraceptives); 394 students were included in the analysis of the prevalence of hyperandrogenic states based on medical examination plus blood sampling (104 were excluded due to active treatment with oral contraceptives and 21 because of thyroid dysfunction or hyperprolactinemia) (Figure 1 and Table 1).

The prevalence of the hyperandrogenic state slightly varied in subjects accepting or refusing to provide blood

Table 2. Selected Characteristics in the Group of Subjects Whose Clinical State Was Assessed by Medical Examination (n = 835; See Table 1)

Variables	Normal (n = 560)	Isolated Menstrual Irregularity (n = 109)	Isolated Clinical Hyperandrogenism (n = 134)	Menstrual Irregularity + Clinical Hyperandrogenism (n = 32)	*P* Value[a]
Mean age, y (SD)	17.4 (1.0)	17.3 (1.0)	17.4 (1.0)	17.7 (1.0)	
High school type, %					
Lyceum	52.3	54.1	60.5	40.6	
Technical institute	23.8	23.9	19.4	31.3	
Professional institute	23.9	22.0	20.1	28.1	
Mean age at menarche, y (SD)	12.3 (1.2)	12.7 (1.4)	11.8 (1.3)	12.3 (1.1)	b,c,d
Mean gynecological age, y (SD)	5.1 (1.6)	4.7 (1.8)	5.6 (1.5)	5.4 (1.4)	b,c,d
Mean weight, kg (SD)	59.3 (10.3)	58.1 (11.4)	61.1 (10.3)	61.1 (8.6)	
Mean BMI (SD)	22.0 (3.6)	21.5 (3.8)	22.7 (3.5)	22.9 (3.0)	
Mean waist circumference (SD), cm	76.0 (9.0)	74.7 (9.2)	76.8 (9.8)	77.1 (8.0)	
Mean waist to hip ratio (SD)	0.8 (0.06)	0.8 (0.06)	0.8 (0.09)	0.8 (0.06)	
Mean systolic blood pressure, mm Hg (SD)	114.3 (13.3)	114.8 (11.6)	115.6 (12.6)	113.3 (12.3)	
Mean diastolic blood pressure, mm Hg (SD)	69.2 (8.8)	70.1 (9.2)	70.2 (9.1)	69.1 (7.9)	
Fatty mass, % (SD)	22.1 (8.8)	21.5 (9.2)	21.5 (8.7)	22.3 (7.9)	
Acanthosis, %	5.4	4.6	11.9	12.5	c,d

[a] χ^2 test was used for categorical variables; 1-way ANOVA with Sidak corrections was used for continuous ones.

[b] $P < .05$ for the comparison between normal subjects and subjects with isolated menstrual irregularity.

[c] $P < .05$ for the comparison between normal subjects and subjects with isolated clinical hyperadrogenism.

[d] $P < .05$ for the comparison between normal subjects and subjects with both menstrual irregularity and clinical hyperandrogenism.

samples, but such differences were never significant; thus, the 2 groups can be considered together in making inferences from the results (Table 1). Therefore, considering the correct diagnosis definition (after questionnaire plus medical examination and excluding the subjects who were taking oral contraceptives), isolated menstrual irregularity was found in 13.0% of the sample, isolated clinical hyperandrogenism in 16.1%, and both states in 3.8% (Table 1). In the subsample of students for whom laboratory tests could be performed, and T levels could therefore be assessed (n = 394; Table 1), approximately 10% of students defined as normal with medical examination showed abnormally high levels of T and were therefore subsequently diagnosed as affected by isolated hyperandrogenemia (6.6% of the sample). In addition, a minority (7.2%) of students defined with isolated clinical hyperandrogenism during the medical examination also showed high T levels (1.3% of the sample). A total of 4.3% of the sample proved to be affected by PCOS.

Predictors of hyperandrogenic state

At the initial univariate analysis in which the distributions of all recorded variables after the medical examination (Table 2) or after all phases of the study (Table 3) were compared across hyperandrogenic state, a few variables showed some significant association with the clinical status: age at menarche, gynecological age, and acanthosis nigricans. The multivariate analyses substan-

Table 3. Selected Characteristics in the Group of Subjects Whose Clinical State Was Assessed by Medical Examination and Blood Sample (n = 394; See Table 1)

Variables	Normal (n = 240)	Isolated Menstrual Irregularity (n = 40)	Isolated Hyperandrogenemia (n = 26)	Isolated Clinical Hyperandrogenism[a] (n = 61)	PCOS (n = 17)	*P* Value[b]
Mean age, y (SD)	17.4 (0.9)	17.4 (0.9)	17.4 (1.1)	17.5 (0.9)	17.6 (0.9)	
High school type, %						
Lyceum	60.4	62.5	57.7	57.8	47.1	
Technical institute	23.8	25.0	26.9	22.5	35.3	
Professional institute	15.8	12.5	15.4	19.7	17.6	
Mean age at menarche (SD), y	12.3 (1.3)	12.5 (1.4)	12.3 (1.3)	11.6 (1.2)	12.1 (0.9)	c,d
Mean gynecological age, y (SD)	5.1 (1.6)	4.9 (1.8)	5.1 (2.1)	6.0 (1.6)	5.4 (1.4)	c,d
Mean weight, kg (SD)	58.6 (9.2)	57.7 (7.9)	63.2 (11.9)	60.2 (10.0)	62.8 (12.7)	
Mean BMI, kg/m^2 (SD)	21.7 (3.1)	21.1 (2.4)	23.3 (4.5)	22.6 (3.3)	23.4 (5.0)	
Mean waist circumference, cm (SD)	75.9 (8.5)	73.8 (7.1)	79.4 (10.3)	76.1 (10.1)	77.0 (10.1)	
Mean waist to hip ratio (SD)	0.8 (0.06)	0.8 (0.05)	0.8 (0.06)	0.8 (0.08)	0.8 (0.06)	
Mean systolic blood pressure, mm Hg (SD)	113.5 (12.8)	114.8 (10.5)	113.7 (13.5)	115.4 (12.4)	116.5 (12.6)	
Mean diastolic blood pressure, mm Hg (SD)	69.3 (9.3)	72.4 (8.7)	70.4 (9.0)	70.8 (8.7)	70.9 (8.7)	
Fatty mass, % (SD)	21.1 (7.9)	20.4 (6.7)	22.3 (9.5)	20.6 (8.4)	23.9 (8.5)	
Acanthosis, %	4.2	5.0	3.9	14.1	11.8	c
Mean total cholesterol, mg/dL (SD)	165.4 (30.5)	165.4 (31.9)	180.5 (32.7)	161.4 (27.0)	170.2 (35.7)	
Mean HDL levels, mg/dL (SD)	59.5 (11.5)	62.3 (11.9)	66.2 (11.2)	60.2 (12.8)	61.8 (15.7)	
Mean triglycerides, mg/dL (SD)	70.5 (42.6)	66.5 (21.2)	68.5 (25.4)	69.0 (30.0)	80.0 (30.7)	
Mean serum glucose, mg/dL (SD)	78.9 (19.5)	77.9 (11.5)	77.2 (15.6)	80.9 (9.5)	72.9 (13.2)	
Fasting insulin level, μU/mL (SD)	8.3 (4.8)	8.6 (3.9)	9.7 (5.6)	10.3 (11.0)	8.1 (4.0)	
HOMA (SD)	1.6 (1.2)	1.7 (0.8)	1.9 (1.4)	2.1 (2.0)	1.5 (0.7)	
QUICKI (SD)	0.16 (0.01)	0.16 (0.02)	0.16 (0.01)	0.16 (0.02)	0.16 (0.01)	

All *P* values that are not indicated were greater than .05.

[a] Five subjects had clinical hyperandrogenism combined with hyperandrogenemia.

[b] χ^2 test was used for categorical variables; 1-way ANOVA with Sidak corrections was used for continuous ones.

[c] $P < .05$ for the comparison between normal subjects and subjects with isolated clinical hyperandrogenism.

[d] $P < .05$ for the comparison between subjects with isolated menstrual irregularity and subjects with isolated clinical hyperandrogenism.

tially confirmed univariate analyses: as compared with normal subjects, lower gynecological age (or higher age at menarche; data not shown because of multicollinearity) was associated with a significantly higher likelihood of isolated menstrual irregularity (OR 0.79; $P = .007$; Table 4); the opposite trend was observed for isolated clinical hyperandrogenism (OR of gynecological age 1.29; $P = .002$); finally, subjects with acanthosis nigricans were more likely to show isolated clinical hyperandrogenism (OR 2.23; $P = .029$). Again, the results of the multivariate analyses, stratified by level of the participation substantially, confirmed the results based on the overall sample (Table 4).

Finally, at the multivariate analysis restricted to the students participating in all phases of the study, in which also laboratory variables could be included in the polytomous logistic regression model (Table 5), the association between higher gynecological age (OR 1.37; $P = .001$) and acanthosis nigricans (OR 4.03; $P = .016$) with isolated clinical hyperandrogenism was confirmed. In addition, some other variables were found to be significantly related with some hyperandrogenic states: higher BMI (OR 1.26; $P = .033$) and high-density lipoprotein cholesterol level (OR 1.06; $P = .001$) were associated with a higher likelihood of isolated hyperandrogenemia. In addition, higher BMI also increased the likelihood of PCOS (OR 1.42; $P = .009$), and larger waist circumference was associated with a lower probability of isolated clinical hyperandrogenism (OR 0.95; $P = .039$).

The 55 women with acanthosis nigricans among the 835 women with a medical visit (Table 2) showed a significantly higher BMI than the other women: 27.4 kg/m^2 (SD 4.8) vs 21.7 (SD 3.15), respectively ($P < .001$). This difference remained highly significant, even after adjusting for HOMA-IR, QUICKI, and T level (in a linear regression model predicting BMI, the regression coefficient for women with vs without acanthosis nigricans was 4.91; 95% confidence interval 3.87–5.95, $P < .001$). On the contrary, although HOMA-IR values were significantly higher in women with acanthosis nigricans (2.61 ± 1.30 vs 1.69 ± 1.34, $P < .001$) at the univariate analysis, such a difference was no longer significant when BMI was also

Table 4. Multivariate Analyses Predicting Hyperandrogenic State (Defined on the Basis of Medical Examination)

Variables	Isolated Menstrual Irregularity OR (95% CI)	*P* Value	Isolated Clinical Hyperandrogenism OR (95% CI)	*P* Value	Menstrual Irregularity + Clinical Hyperandrogenism OR (95% CI)	*P* Value
Age, 1-y increase	1.16 (0.88–1.53)[a]	.3	0.79 (0.61–1.03)[a]	.077	1.37 (0.84–2.24)[a]	.2
	1.11 (0.77–1.62)[b]	.5	0.88 (0.61–1.28)[b]	.5	1.77 (0.97–3.24)[b,c]	.064
	1.20 (0.79–1.83)[d]	.4	0.71 (0.48–1.03)[d]	.071	0.80 (0.33–1.93)[c,d]	.6
Gynecological age, 1-y increase	0.79 (0.67–0.94)[a]	.007	1.29 (1.10–1.52)[a]	.002	0.96 (0.71–1.29)[a]	.7
	0.75 (0.59–0.95)[b]	.015	1.03 (0.81–1.30)[b]	.8	0.82 (0.56–1.18)[b,c]	.2
	0.83 (0.64–1.08)[d]	.16	1.54 (1.23–1.92)[d]	.071	1.26 (0.76–2.09)[c,d]	.3
Mean BMI (SD)	1.00 (0.9–1.1)[a]	.9	1.04 (0.95–1.14)[a]	.4	1.10 (0.93–1.30)[a]	.3
	0.98 (0.85–1.13)[b]	.8	1.01 (0.88–1.15)[b]	.8	1.02 (0.83–1.28)[b,c]	.8
	1.00 (0.85–1.18)[d]	.9	1.03 (0.9–1.19)[d]	.6	1.20 (0.88–1.64)[c,d]	.2
Waist circumference, 1-cm increase	0.98 (0.95–1.02)[a]	.4	0.98 (0.95–1.01)[a]	.3	0.97 (0.91–1.04)[a]	.4
	0.99 (0.94–1.06)[b]	.9	1.01 (0.96–1.07)[b]	.6	1.00 (0.92–1.10)[b,c]	.8
	0.97 (0.92–1.03)[d]	.4	0.96 (0.91–1.00)[d]	.10	0.93 (0.83–1.05)[c,d]	.2
Systolic blood pressure, 1-mm Hg increase	1.00 (0.99–1.02)[a]	.4	1.00 (0.99–1.02)[a]	.7	0.99 (0.96–1.02)[a]	.5
	1.00 (0.98–1.02)[b]	.9	1.00 (0.98–1.02)[b]	.9	0.98 (0.95–1.02)[b,c]	.4
	1.02 (0.99–1.04)[d]	.2	1.00 (0.99–1.03)[d]	.4	1.00 (0.95–1.06)[c,d]	.8
Acanthosis, yes vs no	1.08 (0.38–3.03)[a]	.9	2.23 (1.08–4.60)[a]	.029	2.40 (0.67–8.43)[a]	.2
	0.55 (0.12–2.62)[b]	.5	1.15 (0.39–3.32)[b]	.8	2.35 (0.54–10.30)[b,c]	.2
	2.05 (0.47–8.86)[d]	.3	4.43 (1.49–13.2)[d]	.007	1.89 (0.14–25.5)[c,d]	.6

Abbreviation: CI, confidence interval. For all ORs and relative 95% CIs, the reference category was normality. The following covariates were also tested for significance: age at menarche, weight, waist to hip ratio, percentage of fatty mass, and diastolic blood pressure. None of them was significant, and they were not included in the final model because of multicollinearity with 1 or more of the covariates reported in the table.

[a] Polytomous logistic regression model including all subjects who compiled the questionnaire and were examined and excluding those who were on active treatment with oral contraceptives (n = 835; see Table 1).

[b] Polytomous logistic regression model including only those subjects who compiled the questionnaire and were examined but refused further participation and excluding those who were on active treatment with oral contraceptives (n = 420; see Table 1).

[c] The number of subjects (successes) in this group was very small (fewer than 30): the lack of significance for some covariates could be due to an insufficient statistical power, and the results should be interpreted with caution (see text for more details).

[d] Polytomous logistic regression model including only those subjects who compiled the questionnaire were examined and provided blood sample and excluding those who were on active treatment with oral contraceptives (n = 415; see Table 1).

Table 5. Multivariate Analyses Predicting Hyperandrogenic State (Defined on the Basis of Medical Examination and Blood Sampling)

Variables	Isolated Menstrual Irregularity OR (95% CI)	*P* Value	Isolated Hyperandrogenemia OR (95% CI)[a]	*P* Value	Isolated Clinical Hyperandrogenism OR (95% CI)[b]	*P* Value	PCOS OR (95% CI)[a]	*P* Value
Gynecological age, 1-y increase	0.88 (0.70–1.11)	.3	0.93 (0.71–1.23)	.6	1.37 (1.14–1.64)	.001	1.04 (0.75–1.46)	.8
Mean BMI (SD)	1.00 (0.83–1.22)	.9	1.26 (1.01–1.59)	.033	1.12 (0.97–1.29)	.13	1.42 (1.09–1.84)	.009
Waist circumference, 1-cm increase	0.97 (0.91–1.04)	.3	1.00 (0.93–1.08)	.9	0.95 (0.90–0.99)	.039	0.91 (0.83–1.00)	.060
Acanthosis, yes vs no	2.70 (0.46–15.3)	.3	0.36 (0.03–4.04)	.4	4.03 (1.30–12.6)	.016	1.65 (0.19–14.5)	.6
HDL cholesterol, 1-U increase	1.02 (0.99–1.06)	.12	1.06 (1.03–1.10)	.001	1.00 (0.98–1.03)	.6	1.03 (0.99–1.08)	.2
Glucose levels, 1-mg/dL increase	1.00 (0.97–1.02)	.7	0.98 (0.95–1.01)	.3	1.00 (0.99–1.02)	.4	0.97 (0.92–1.00)	.052

Abbreviations: CI, confidence interval; HDL, high-density lipoprotein. Polytomous logistic regression model included all subjects who compiled the questionnaire were examined and provided blood samples and excluded those who were on active treatment with oral contraceptives and were diagnosed with thyroid dysfunction or hyperprolactinemia (n = 394; see Table 1). For all ORs and relative 95% CIs, the reference category was normality. The following covariates were also tested for significance: age at menarche, weight, waist to hip ratio, percentage of fatty mass, diastolic blood pressure, total cholesterol, triglycerides, fasting insulin, HOMA, and QUICKI. None of them was significant, and they were not included in the final model because of multicollinearity with 1 or more of the covariates reported in the table. Age (nonsignificant in all models) was also excluded because of the relatively scarce sample size to reduce the likelihood of overfitting.

[a] The number of subjects (successes) in this group was very small (fewer than 30): the lack of significance for some covariates could be due to an insufficient statistical power, and the results should be interpreted with caution (see text for more details).

[b] Five subjects had clinical hyperandrogenism combined with hyperandrogenemia.

adjusted for. Similarly, QUICKI was significantly lower among women with acanthosis nigricans (0.15 ± 0.01 vs 0.16 ± 0.01, $P < .001$), but the significance was lost after controlling for BMI. Finally, T levels were not significantly different in women with or without acanthosis nigricans at both the univariate and multivariate analysis ($P = .7$).

Discussion

This study provides for the first time a reliable assessment of the prevalence of hyperandrogenic states in Italian late adolescent and young females aged 16–19 years and indicates that hyperandrogenic disorders are very prevalent in these women from a young age. In fact, we found that the prevalence rates were approximately 10% for isolated menstrual irregularity; 17% for isolated clinical hyperandrogenism (mainly represented by hirsutism); 7% for hyperandrogenemia, isolated, or combined with clinical hyperandrogenism; and 4% for PCOS.

Although the overall prevalence of each hyperandrogenic state did not significantly differ among the 3 levels of participation, this study clearly demonstrates that self-reports cannot be trusted at an individual level for some clinical states. In particular, we found that the questionnaire had a high level of reliability in identifying normality and isolated menstrual irregularity but a low level of reliability in identifying clinical hyperandrogenism. This result, in agreement with the study by Wild et al (29), suggests that self-scoring hyperandrogenism is not a reliable index of hyperandrogenic states and led us to conclude that the validity of studies based only on this methodology to grade hirsutism or alopecia may be largely imprecise. In the present study, hirsutism and alopecia were evaluated and scored by the same well-trained physician; this obviously increases the reliability of the detection and therefore gives strength to the study. Another strong point of this study is the use of LC-MS/MS to measure T, a method with good precision, sensitivity, and high accuracy for circulating female T levels (19, 30–32). In addition, a reference interval of T suitable for the age of the study sample was created. This adds validity to the results because a well-known physiological increase in androgen levels during adolescence and youth is present (12). Consequently, the use of adult normative data to assign young females to normal or high androgen status, as performed in previous studies (33, 34), should be considered with caution.

Data on prevalence of hyperandrogenic states that emerge from this study differ from those described in the only community-based study on predominantly Caucasian female adolescents performed so far (9). In particular, we found a lower prevalence of menstrual irregularity (10% vs 52%), a higher prevalence of clinical hyperandrogenism (17% vs 8%), and a slightly higher prevalence of PCOS using National Institutes of Health diagnostic criteria (4% vs 3%). The large differences in terms of menstrual irregularity and clinical hyperandrogenism between the 2 populations can be the consequence of the higher gynecological age (time since menarche) of the 394 students of our study with both medical examination and blood sampling with respect to the 244 students studied by

Hickey at al (9) (minimum and mean time since menarche: 24 vs 2 months and 64 vs 32 months, respectively). In fact, most adolescents establish regular cycles by 2 years after menarche (65% after the first year), and additionally, hirsutism takes time to develop in the presence of increased androgens (12). Other factors may obviously contribute, such as metabolism, environment, and diet, whereas anthroprometry does not seems to be involved because it did not differ between the 2 populations. The slightly higher prevalence of PCOS in our population could also derive from the assay used to measure testosterone (LC-MS/MS vs RIA), which is the most accurate method at low T blood levels (19).

The design of the study allowed us to define several predictive factors of hyperandrogenic states in these young women. Of great interest was the finding that acanthosis nigricans was associated with a significantly higher likelihood of isolated clinical hyperandrogenism and that higher BMI increased the likelihood of isolated high T levels and PCOS. Acanthosis nigricans is known as a good cutaneous marker for insulin resistance (35) and is probably more accurate than HOMA-IR and QUICKI in screening large populations. HOMA-IR and QUICKI are simple fasting measures of insulin sensitivity that demonstrate good accuracy when compared with the euglycemic hyperinsulinemic clamp technique, which represents the current gold standard for assessing insulin sensitivity in humans, in small and fairly homogeneous populations (21, 22). QUICKI, which incorporates both inversion and logarithmic transformations of fasting glucose and fasting insulin appears to be more accurate than HOMA-IR in insulin-resistant subjects and therefore in obese and type 2 persons with diabetes (22). Nevertheless, both these methods lose some of their accuracy when applied to cohorts with a broad range of insulin insensitivity (22) as found in epidemiological studies. In these circumstances, acanthosis nigricans has been found to be better in screening subjects for insulin resistance and identifying subjects at risk for developing type 2 diabetes (36). In any case, these data allowed us to conclude that what has been previously observed in adults (37) may also apply to young females, in particular that insulin resistance (and compensatory hyperinsulinemia) may exert a trigger role in determining the appearance of hyperandrogenic disorders.

Acanthosis nigricans was also observed in 5% of our young population defined as normal for hyperandrogenic disorders; this percentage, in line with the literature (18), probably reflects the known physiological and transitional increase of insulin resistance appearing during puberty and characterizing even late adolescence (38).

In addition, the association of higher BMI with hyperandrogenemia and PCOS was expected, thus confirming previous data showing that weight excess may play an important role in the expression of hyperandrogenic disorders, particularly PCOS, probably through the increment of circulating testosterone availability (7, 39).

In conclusion, this study confirms that hyperandrogenic disorders, including PCOS, have their origin during adolescence and young age, thus supporting the need to focus more research efforts on young age ranges and consequently to promote the knowledge about predictors, thus providing recommendations for clinical management, and clearly defining specific diagnostic criteria.

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