Ovarian Function during Puberty in Girls with Type 1 Diabetes Mellitus: Response to Leuprolide

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Context: An increased prevalence of polycystic ovary syndrome (PCOS) has been reported in adult women with type 1 diabetes mellitus (DM1). We investigated whether these hormonal abnormalities begin during puberty by evaluating the ovarian steroidogenic response to leuprolide acetate.

Methods: We studied 56 adolescent girls with DM1 (aged 12.3 ± 0.2 yr) and 64 healthy girls (C) (aged 11.9 ± 0.2 yr) up to 2 yr post menarche, matched by age, body mass index, and pubertal development. We evaluated anthropometrical data and Ferriman-Gallway score and performed a leuprolide test (500 μg sc) to study ovarian function. Ovarian volume was determined by transabdominal ultrasonography.

Results: We found five DM1 but no C girls with abnormally located terminal hair (Fisher's exact, $P < 0.05$). Free androgen index increased throughout puberty in girls with DM1 (ANOVA, $P < 0.0001$), which was associated with a decrease in SHBG levels in girls with DM1 (ANOVA, $P < 0.0001$). Stimulated 17OH progesterone (17OHProg) increased throughout puberty only in girls with DM1 (ANOVA, $P < 0.01$). Girls with DM1 at Tanner stage 5 had higher stimulated LH to FSH ratio, testosterone, and 17OHProg levels than girls at Tanner stage 4. In contrast, in C girls the stimulated testosterone, 17OHProg, and LH to FSH ratio were similar at Tanner stages 4 and 5. Ovarian volumes and uterine length were larger in girls with DM1 (analysis of covariance, $P < 0.05$).

Conclusions: These data suggest that patients with DM1 have differences in ovarian steroidogenic response to leuprolide, compared with C girls during puberty. Future studies in young women should clarify whether these findings are related to the pathogenesis of hyperandrogenism later in life

POLYCYSTIC OVARY SYNDROME (PCOS) is frequently associated with insulin resistance and type 2 diabetes mellitus (1). Recently an increased prevalence of PCOS has been observed in adult women with type 1 diabetes mellitus (DM1) as well. Escobar-Morreale *et al.* (2) evaluated 68 adult women with DM1 and observed that 18.8% had PCOS and 20% had hirsutism without other endocrinological abnormalities. An onset of DM1 before menarche was a risk factor for the subsequent development of hyperandrogenic disorders (2), suggesting that the presence of DM1 during adolescence may play a critical role in the pathogenesis of hyperandrogenism.

It has been suggested that the use of exogenous insulin to treat DM1 in these patients may contribute to the development of PCOS. Insulin is administered in a nonphysiological fashion because it is injected sc and is absorbed into the systemic circulation (3), potentially stimulating the synthesis of androgens by the ovaries (4). *In vitro* studies have shown that insulin acts synergically with LH to stimulate the synthesis of testosterone by ovarian thecal cells (4). An additional pathogenic mechanism might be the exacerbated insulin resistance observed in patients with DM1 during puberty (5), which has been proposed to play a role in the pathogenesis of PCOS (6).

PCOS is the result of an abnormal regulation of steroidogenesis, specifically of androgen secretion by the ovary. Provocative tests using GnRH analogs have shown that the hyperandrogenism has an ovarian source in most cases (7, 8). Such alteration may precede the development of clinical manifestations of PCOS and has been termed functional ovarian hyperandrogenism (FOH) (8).

Clinical manifestations of PCOS usually arise during the perimenarchal period (9), but earlier manifestations of this syndrome, such as precocious pubarche, have been observed before puberty (10). It is not known, however, whether a similar sequence of events occurs in girls with DM1. Therefore, the purpose of this study was to determine whether the hyperandrogenism described in some adult women with DM1 is already detectable during puberty. With this aim, we evaluated the hormonal response to a GnRH analog in a rather large group of pubertal girls with DM1 and compared their response with a carefully matched group of normal girls in a cross-sectional study.

Abbreviations: ANCOVA, Analysis of covariance; BMI, body mass index; C, control girls; CV, coefficient of variation; DHEAS, dehydroepiandrosterone sulfate; DM1, diabetes mellitus type 1; FAI, free androgen index; FOH, functional ovarian hyperandrogenism; HbA1c, hemoglobin A1c; 17OHProg, 17OH progesterone; PCOS, polycystic ovary syndrome.

Subjects and Methods

Subjects

Pubertal girls (n = 56) with DM1 were recruited from four public hospitals in Santiago, Chile. Girls had breast development Tanner stages

2–5 (up to 2 yr post menarche), persistent insulinopenia, and a C-peptide level 0.05 nmol/liter or less. Exclusion criteria were specific types of diabetes mellitus; type 2 diabetes mellitus; honeymoon period; abnormal thyroid function; use of sex steroids; and presence of other concomitant chronic conditions such as genetic syndromes, celiac disease, renal, liver, or cardiac disease, or undernourishment. All girls were receiving intermediate (NPH) and soluble (either regular or lispro) insulin in two (39.8%) or more (60.2%) daily injections. Daily insulin doses used during the last 15 d before study as well as mean hemoglobin A1c (HbA1c) levels during the last year were recorded.

Healthy girls (C) were recruited from nearby schools matched by socioeconomic status (n = 64). Inclusion criteria were breast Tanner stages 2–5 (up to 2 yr post menarche), normal fasting glucose, and absence of any chronic diseases. The DM1 and C girls were matched according to Tanner stage, chronological age, and body mass index (BMI).

Study protocol

A complete physical examination was performed by one of the authors (E.C. or N.U.). Pubertal development was assessed according to Marshall and Tanner (11). Hirsutism was evaluated by determining the presence of terminal hair using the modified Ferriman-Gallway score (12, 13). The Chilean population is less hirsute than other populations, so a score 5 or greater was employed to determine the presence of hirsutism (14); such a score has been suggested to be pathological in young adolescents (15). The presence of acne was also determined. Menstrual regularity was not evaluated because irregular menses are physiologic in the first years after menarche, so we decided to exclude this observation as a sign of hyperandrogenism.

Weight was measured using a conventional Seca scale with a precision of 100 g and height was measured with a Harpenden stadiometer. Waist circumference was measured to the nearest 0.5 cm, using a flexible measuring tape at the narrowest circumference between the lower costal margin and the iliac crest in the standing position. The hip circumference measurement was obtained at the maximum perimeter at the level of the femoral trochanters. Waist to hip ratio was calculated as the ratio of these two circumferences. SD scores were calculated for height, weight, and BMI using current National Center for Health Statistics standard curves (16).

Girls underwent a leuprolide test with 500 μg leuprolide acetate injected sc as previously described (17). The test was started between 0800 and 0900 h, and blood samples were obtained before and 3 and 24 h after the injection. Testosterone, androstenedione, 17OH progesterone (17OHProg), and dehydroepiandrosterone sulfate (DHEAS) were analyzed in the basal and 24-h samples. SHBG was measured in the basal sample and the free androgen index (FAI) was calculated from the formula 100 × testosterone (nanomoles per liter)/SHBG (nanomoles per liter) (18). In postmenarchal girls, the test was performed during the follicular phase between d 3 and 8.

Transabdominal ultrasonography was performed by a single observer (H.S.) with a 5-MHz transducer in a Sonoace 6000C equipment (Medison Co., Seoul, Korea). Ovarian volume was calculated using the simplified formula for a prolate ellipsoid (19). The larger ovary was used to evaluate ovarian size.

The protocol was approved by the respective institutional review boards. All parents signed informed consents and girls gave their assent before entering the study.

Hormone assays

Serum testosterone, androstenedione, 17OHProg, and DHEAS were measured by competitive specific binding RIAs (Diagnostic System Laboratories, Webster, TX); interassay coefficients of variation (CVs) were 8.1, 8.9, 7.3, and 7.7%, respectively; intraassay CVs were 5.3, 4.2, 7.7, and 5.3%, respectively (20). HbA1c levels were measured using a commercially available automatic system (DCA 2000, Bayer Diagnostics, Tarrytown, NY).

Serum LH, FSH, and SHBG levels were measured by immunoradiometric assays from Diagnostic System Laboratories. Intraassay CVs were 6.5% for LH, 3.6% for FSH, and 3.9% for SHBG. Interassay CVs were 7.6% for LH, 6.2% for FSH, and 6.9% for SHBG.

Serum estradiol, insulin, and C-peptide levels were determined by RIAs as previously described (20, 21)

Statistical analysis

Clinical and laboratory data are shown as mean ± SEM. Comparisons of means between the two groups (girls with DM1 and C) for each Tanner stage were performed using the Mann-Whitney *U* test. Differences within each group among the various Tanner stages were assessed by one-way ANOVA, followed by the least significant differences test for multiple comparisons. Interaction between Tanner stage and group was estimated by full-fact analysis of covariance (ANCOVA). The effect of BMI, insulin doses, and metabolic control on SHBG and stimulated 17OHProg levels was also evaluated using ANCOVA. Duration of disease was not included in the analyses because it showed strong colinearity with age and Tanner stage. Differences in proportions between the two groups were evaluated using Fisher's exact test. Results are expressed as mean age ± SEM. All statistic calculations were run on SPSS for Windows (version 10.0; SPSS, Inc., Chicago, IL). $P < 0.05$ was considered statistically significant.

Results

Fifty-six girls with DM1 (aged 12.3 ± 0.2 yr) and 64 C girls (aged 11.9 ± 0.2 yr) were studied. Their clinical characteristics are shown in Table 1. The proportion of postmenarchal girls in each group was similar (20 girls with DM1 and 14 C girls, $P > 0.05$), and their gynecological age was 1.2 ± 0.2 yr in girls with DM1 and 1.1 ± 0.2 yr. in C ($P = 0.86$, Mann-Whitney *U* test).

Six girls with DM1, but no C, had a Ferriman-Gallway score equal to or greater than 5 (range 5–8, Fisher's exact test,

TABLE 1. Clinical and anthropometric characteristics of girls with DM1 and C as well as metabolic control in girls with DM1 according to Tanner stage (T)

	Tanner 2		Tanner 3		Tanner 4		Tanner 5	
	DM1	C	DM1	C	DM1	C	DM1	C
n	14	15	14	15	12	15	16	19
Age (yr)	10.8 ± 0.3	10.4 ± 0.3	11.7 ± 0.2	11.3 ± 0.3	12.6 ± 0.3	12.1 ± 0.3	13.9 ± 0.3	13.4 ± 0.2
BMI SDS	−0.2 ± 0.3	0.4 ± 0.2	0.1 ± 0.2	0.3 ± 0.4	0.3 ± 0.2	0.7 ± 0.2	0.8 ± 0.1	0.8 ± 0.2
Height SDS	−0.5 ± 0.3	−0.4 ± 0.3	−0.3 ± 0.3	−0.4 ± 0.3	0.0 ± 0.3	0.3 ± 0.2	−0.5 ± 0.3	−0.3 ± 0.2
Waist to hip ratio	80.1 ± 1.3	83.7 ± 1.3	78.8 ± 1.2[a]	83.3 ± 1.2	81.7 ± 1.8	82.1 ± 1.3	79.0 ± 1.3	80.2 ± 1.0
DM1 duration (yr)	4.0 ± 0.7		3.3 ± 0.8		4.8 ± 1.0		5.1 ± 0.8	
HbA1c (%)	8.9 ± 0.5		8.0 ± 0.4		9.9 ± 0.6[b]		9.2 ± 0.5	
Insulin dose (U/kg·d)	0.9 ± 0.1		0.8 ± 0.1		1.2 ± 0.1[c]		1.0 ± 0.1	

Data are shown as mean ± SEM. SDS, SD score.

[a] $P < 0.05$ DM1 T3 *vs.* C T3 (Mann-Whitney *U*).

[b] $P < 0.05$ DM1 T4 *vs.* DM1 T3 (ANOVA).

[c] $P < 0.05$ DM1 T4 *vs.* DM1 T2–3 and $P < 0.01$ DM1 T4 *vs.* DM1 T2 (ANOVA).

$P < 0.01$). In contrast, all C girls showed Ferriman-Gallway scores of either 0 or 1. The age range of the hirsute DM1 patients was 8.5–14.3 yr, two were in Tanner 2, and the remaining were in Tanner 5. Three of the hirsute DM1 girls exhibited increased testosterone levels and FAI. Acne was observed in a similar proportion in both groups of girls (11 girls in each group had some acne, being moderate to severe in three DM1 girls and two C, $P > 0.05$). Seven C and two DM1 girls had a family history of PCOS, but the clinical and laboratory findings were similar whether these patients were included or excluded. Thus, it was decided to include these patients in the final analyses.

Gonadotropins (Table 2 and Fig. 1). Basal FSH levels increased throughout puberty only in DM1 girls (ANOVA, $P < 0.05$). Basal LH to FSH ratio increased in both C and DM1 girls during puberty. Stimulated LH to FSH also increased in both groups up to Tanner stage 4, but this ratio increased further in Tanner 5 DM1 girls only (6.8 ± 2.1 *vs.* 2.5 ± 0.4, ANOVA, $P < 0.05$). In contrast, the stimulated LH to FSH ratio was similar in Tanner 4 and 5 C girls.

Steroids and SHBG. Basal steroid levels are shown in Table 2. FAI behaved similarly in both groups until Tanner stage 4. However, between Tanner 4 and 5, only DM1 girls exhibited a significant increase in FAI (ANOVA, $P < 0.0001$), Tanner stage 5 girls showing significantly higher FAI than girls at Tanner stage 2–4. SHBG decreased during puberty only in girls, with DM1 (ANOVA, $P < 0.0001$) being higher in DM1 than in C at Tanner stages 2 and 3 (Mann Whitney *U*, $P < 0.0001$). Accordingly, ANCOVA showed DM1 to be a significant factor in determining SHBG levels (ANCOVA, $P = 0.001$) (Table 2). In girls with DM1, SHBG levels were significantly related to Tanner stage (ANCOVA, $P < 0.001$) and BMI (ANCOVA, $P = 0.05$) but not insulin dose (per kilogram body weight) or HbA1c levels. We observed no differences in basal serum testosterone levels between girls with DM1 and C, but DHEAS levels were higher in Tanner stage 4 DM1 girls than C at the same stage (Mann Whitney *U*, $P < 0.05$).

Basal and stimulated estradiol levels increased through puberty only in C girls (ANOVA, both $P < 0.01$). Stimulated estradiol levels were higher in C than DM1 girls at Tanner stage 4 (Mann-Whitney *U*, $P < 0.05$)

Steroids levels after the administration of leuprolide acetate are shown in Fig. 2. Stimulated testosterone levels were higher in girls with DM1 at Tanner stage 5 than those at Tanner 2–4 (ANOVA, $P < 0.05$). Accordingly, stimulated 17OHProg was significantly higher in girls with DM1 at Tanner stage 5 than at Tanner stages 2–4 (T5 *vs.* T2: $P < 0.0001$, T5 *vs.* T3: $P < 0.01$, T5 *vs.* T4: $P < 0.05$). In contrast, stimulated 17OHProg and testosterone levels did not increase throughout puberty in C girls. Stimulated and basal 17OHProg were higher in Tanner 2 and 4 C girls than girls with DM1 (Mann-Whitney *U*, both $P < 0.05$). ANCOVA indicated that the interaction between Tanner stage and diabetes on stimulated 17OHProg was significant ($P < 0.01$). When DM1 girls were analyzed separately, stimulated levels of 17OHProg were related to Tanner stage only (ANCOVA, $P < 0.01$) but not to BMI, insulin dose, or metabolic control. Stimulated A2 levels increased through puberty in both groups, and were higher in DM1 than C at Tanner stage 2 only (Mann-Whitney *U*, $P < 0.05$).

Transabdominal ultrasonography (Fig. 3). Ovarian volume, corrected for Tanner stage, was greater in DM1 than C girls (ANCOVA, $P < 0.05$). Uterine length increased through puberty in DM1 and C girls, being larger in DM1 girls, even after adjusting for Tanner stage (ANCOVA, $P < 0.05$).

TABLE 2. Basal steroids and gonadotropins in girls with DM1 and controls (C) according to Tanner stage (T)

	Tanner 2		Tanner 3		Tanner 4		Tanner 5	
	DM1 (n = 14)	C (n = 15)	DM1 (n = 14)	C (n = 15)	DM1 (n = 12)	C (n = 15)	DM1 (n = 16)	C (n = 19)
FAI	1.2 ± 0.2	2.0 ± 0.7	2.2 ± 0.4	4.0 ± 1.1	3.1 ± 0.5	3.7 ± 0.4	6.4 ± 1.1[a]	4.6 ± 0.6
SHBG (nmol/liter)	57.0 ± 2.9[b]	41.3 ± 3.1	50.3 ± 2.9[c]	32.1 ± 3.4	40.6 ± 3.5	38.6 ± 3.6	33.8 ± 3.4[d]	36.9 ± 3.0
Testosterone (ng/dl)	20.3 ± 3.2	17.9 ± 3.5	28.1 ± 4.1	29.1 ± 5.7	33.9 ± 5.4	36.8 ± 2.2	50.8 ± 5.8	43.1 ± 3.8
Androstenedione (ng/ml)	1.2 ± 0.1	0.9 ± 0.1	1.2 ± 0.2	1.3 ± 0.2	1.9 ± 0.2	1.7 ± 0.2	2.1 ± 0.2	1.8 ± 0.1
DHEAS (ng/ml)	518.2 ± 64.3	458.9 ± 67.5	487.0 ± 60.5	520.3 ± 98.4	729.3 ± 93.8[j]	491.3 ± 63.4	876.2 ± 102.2	920.8 ± 97.6
Estradiol (pg/ml)	28.5 ± 6.0	32.5 ± 5.8	39.5 ± 6.5	33.9 ± 3.8	44.0 ± 5.1	57.7 ± 6.8	42.0 ± 3.9	47.2 ± 4.4[e]
FSH (IU/ml)	2.3 ± 0.4	3.1 ± 0.6	4.0 ± 0.7	3.2 ± 0.4	4.7 ± 0.5[f]	4.1 ± 0.3	3.4 ± 0.5	4.2 ± 0.4
LH (IU/ml)	0.6 ± 0.1	0.8 ± 0.2	1.7 ± 0.3	2.0 ± 0.6	2.8 ± 0.4	2.4 ± 0.3	2.1 ± 0.3	2.7 ± 0.4
LH to FSH ratio	0.3 ± 0.1	0.2 ± 0.0	0.4 ± 0.1	0.6 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.9 ± 0.2[g]	0.7 ± 0.1[h]
17OHProg (ng/ml)	0.9 ± 0.1[i]	1.5 ± 0.2	1.0 ± 0.1[i]	1.4 ± 0.1	1.3 ± 0.2	1.3 ± 0.2	1.7 ± 0.3	1.6 ± 0.2

Data are shown as mean ± SE. To convert units to SI: testosterone (nanograms per deciliter) × 0.0347 = nanomoles per liter; androstenedione (nanograms per milliliter) × 3.49 = nanomoles per liter; DHEAS (nanograms per milliliter) × 0.0027 = nanomoles per liter; estradiol (picograms per milliliter) × 3.67 = picomoles per liter; 17OH Prog (nanograms per milliliter) × 3.03 = nanomoles per liter.

[a] $P < 0.0001$ DM1 T5 *vs.* DM1 T2; $P < 0.001$ DM1 T5 *vs.* DM1 T3; $P < 0.01$ DM1 T5 *vs.* DM1 T4 (ANOVA).
[b] $P < 0.0001$ DM1 T2 *vs.* C T2 (Mann-Whitney *U*).
[c] $P < 0.0001$ DM1 T3 *vs.* C T3 (Mann-Whitney *U*).
[d] $P < 0.0001$ DM1 T5 *vs.* T2; $P < 0.0001$ DM1 T5 *vs.* T3 (ANOVA).
[e] $P < 0.05$ C T5 *vs.* C T2 (ANOVA).
[f] $P < 0.05$ DM1 T2 *vs.* DM1 T3; $P < 0.01$ DM T2 *vs.* DM1 T4 (ANOVA).
[g] $P < 0.01$, DM1 T5 *vs.* DM1 T2; $P < 0.05$ DM1 T5 *vs.* DM T3 (ANOVA).
[h] $P < 0.05$ C T5 *vs.* C T2 (ANOVA).
[i] $P < 0.05$ DM1 T2 *vs.* C T2 and DM1 T3 *vs.* C T3 (Mann-Whitney *U*).
[j] $P < 0.05$ DM T4 *vs.* C T4 (Mann-Whitney *U*).

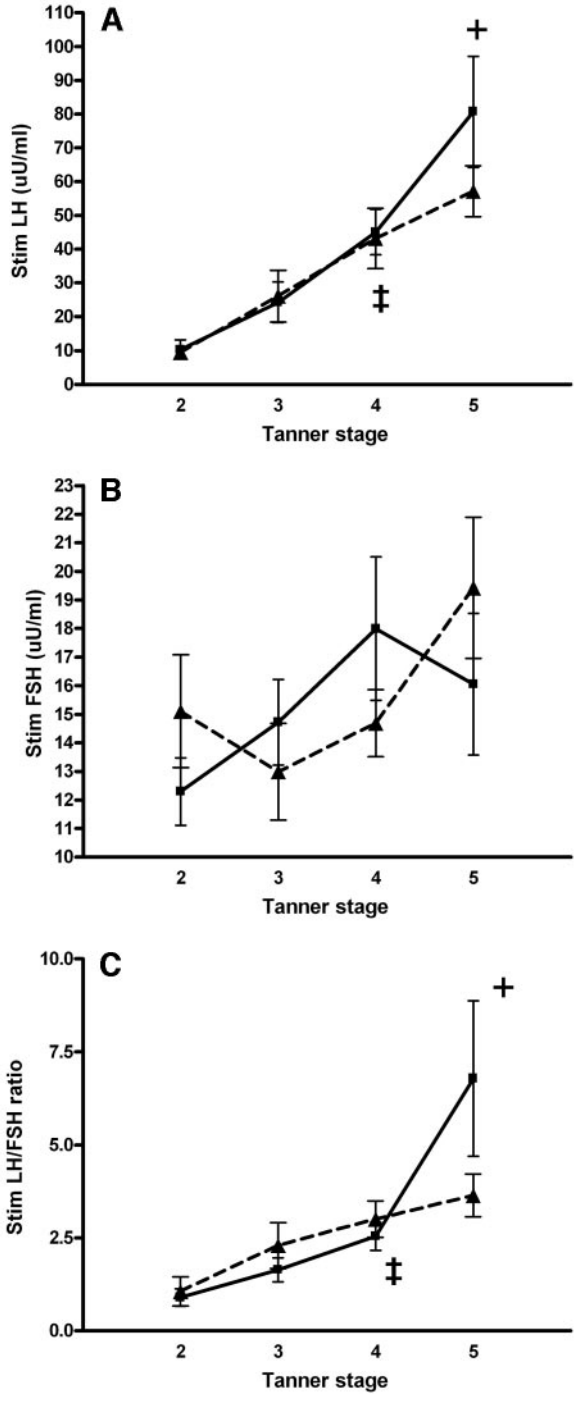

FIG. 1. Gonadotropin levels after the stimuli with leuprolide acetate 500 μg sc in DM1 and C girls according to Tanner stage (T). Stimulated LH, FSH, and LH to FSH ratio were obtained 3 h after the administration of leuprolide acetate. *Broken line* and *triangles*, C; *black line* and *squares*, DM1. Data are shown as average ± SE. A, Stimulated LH levels: +, DM1 T5 *vs.* DM1 T2–4, $P < 0.05$ (ANOVA). ‡, C T4 *vs.* C T2, $P < 0.05$ (ANOVA). B, Stimulated FSH levels. C, Stimulated LH to FSH ratio. +, DM1 T5 *vs.* DM1 T2–4, $P < 0.05$ (ANOVA). ‡, C T4 *vs.* C T2, $P < 0.05$ (ANOVA).

Discussion

We report a comprehensive study of ovarian function in a large group of pubertal girls with DM1, up to 2 yr postmenarche, compared with a control group carefully matched by Tanner stage and BMI. Our data suggests that DM1 may affect ovarian steroidogenic response to leuprolide, a GnRH analog, during puberty, which might play a role in the subsequent development of ovarian hyperandrogenism and PCOS.

It has become increasingly clear that PCOS results from by a dysregulation of steroid biosynthesis, which may become manifested as FOH, defined as an abnormal 17OHProg response to GnRH stimulation (22). In some cases FOH may precede clinical hyperandrogenism (23). Our data show that DM1 may influence the ovarian steroidogenic response to leuprolide acetate, with increasing levels of the 17OHProg throughout puberty. DM1 girls attained maximum 17OHProg levels at the end of puberty, in contrast to C girls who achieved a maximum response to leuprolide acetate during the middle stages of puberty, as previously reported (17). In accordance with our results, Virdis *et al.* (24) found in a group of nine oligomenorrheic postpubertal adolescents with DM1 an elevated 17OHProg response to leuprolide, suggesting the presence of FOH.

In our study, stimulated 17OHProg levels in DM1 girls were not related to BMI, HbA1c, or daily insulin dose. It has been proposed that the increased ovarian steroidogenic activity in women with DM1 may result from the supraphysiological doses of insulin required to achieve optimal metabolic control. Furthermore, exogenous insulin reaches the systemic circulation in a nonphysiological fashion (3) and may bind insulin and IGF-I receptors in the ovary (25, 26). *In vitro*, insulin may act in concert with gonadotropins on the ovary (27) and may also have direct effects on the activity of steroidogenic enzymes. However, it is not known what proportion of the insulin dose administered to girls with DM1 effectively reaches the ovaries. Hence, we cannot exclude an effect of exogenous insulin in our group of girls with DM1.

In addition to an altered response to the leuprolide test, an increase in FAI was observed in girls with DM1. This could be particularly relevant because a recent consensus proposes the FAI as one of the diagnostic criteria for PCOS (28). However, total testosterone levels were similar in DM1 and C at all Tanner stages. Meyer *et al.* (29) found elevated testosterone levels in pubertal girls with DM1 only at Tanner stage 5, which is in accordance with our finding of an increased FAI at the end of puberty in girls with DM1.

The increase in FAI in girls with DM1 may be explained at least in part by decreasing SHBG levels during puberty. ANCOVA showed that this latter finding was related to BMI, as previously described (30, 31), Previous studies have shown that DM1 girls increase their fat mass (32) and their waist to hip ratio (33) during puberty. Although the C and DM1 groups were matched for BMI, we cannot exclude that differences in fat mass associated with DM1 may explain the changes in SHBG levels in our population. It is unlikely that the SHBG levels were affected by insulin dose use, however, because ANCOVA analysis showed that SHBG levels were not related to insulin dose or metabolic control.

Girls with DM1 exhibited increasing FSH levels during puberty. This was not associated with increasing basal or stimulated levels of estradiol, suggesting that ovarian function is influenced by the diabetic condition, as shown in adult

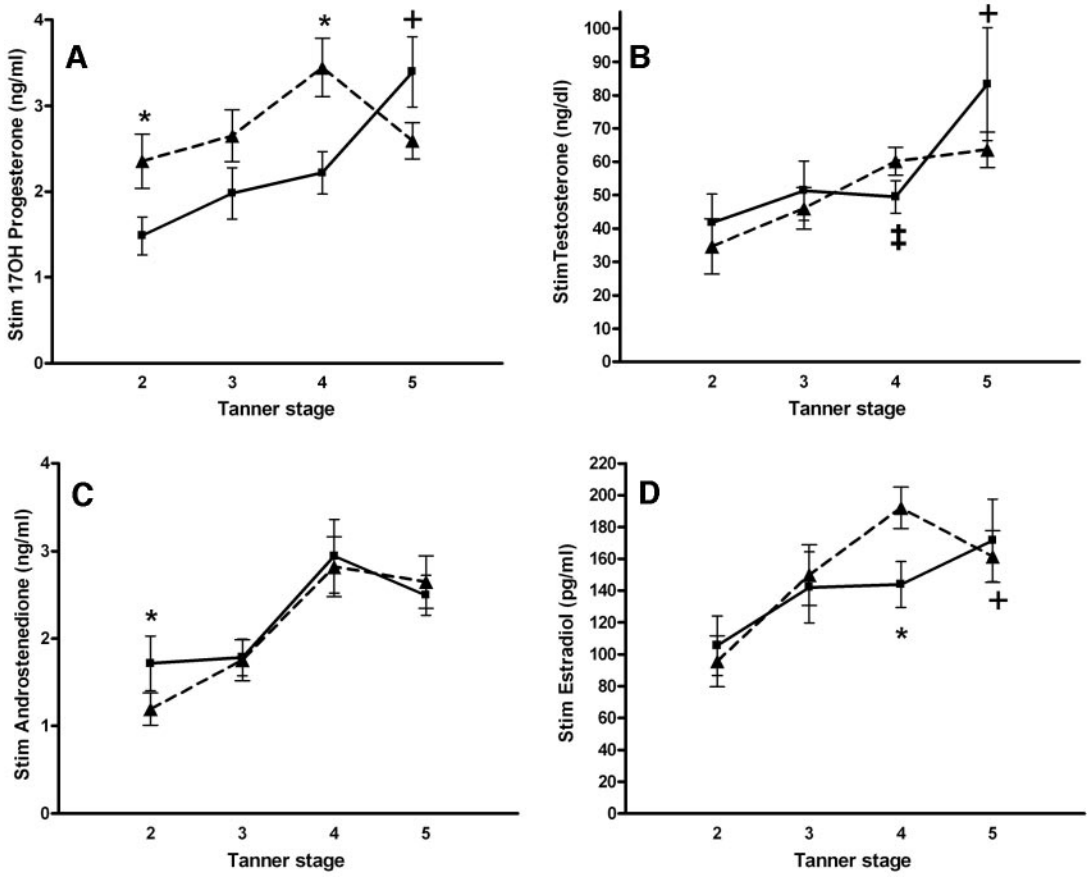

FIG. 2. Steroid levels after the stimulation with leuprolide acetate 500 μg sc in DM1 and C girls according to Tanner stage (T). Stimulated testosterone, 17OHProg, estradiol, and androstenedione were obtained 24 h after the injection with leuprolide acetate. *Broken line* and *triangles*, C girls; *continuous line* and *squares*, DM1 girls. Data are shown as average ± SEM. A, Stimulated 17OHProg levels. +, DM1 T5 *vs.* DM1 T 2–4, $P < 0.05$ (ANOVA). *, DM1 T2 *vs.* C T2, $P < 0.05$ (Mann-Whitney *U*) and DM1 T4 *vs.* C T4, $P < 0.05$ (Mann-Whitney *U*). B, Stimulated testosterone levels. +, DM1 T5 *vs.* DM1 T2–5, $P < 0.05$ (ANOVA). ‡, C T4 *vs.* C T2, $P < 0.05$ (ANOVA). C, Stimulated androstenedione levels. *, DM1 T2 *vs.* C T2, $P < 0.05$ (Mann-Whitney *U*). D, Stimulated estradiol levels. +, C girls showed different estradiol levels at all T stages, $P < 0.01$ (ANOVA). *, DM1 T4 *vs.* C T4, $P < 0.05$ (Mann-Whitney *U*). To convert units to SI: testosterone (nanograms per deciliter) × 0.0347 = nanomoles per liter; androstenedione (nanograms per milliliter) × 3.49 = nanomoles per liter; DHEAS (nanograms per milliliter) × 0.0027 = nanomoles per liter; estradiol (picograms per milliliter) × 3.67 = picomoles per liter; 17OHProg (nanograms per milliliter) × 3.03 = nanomoles per liter.

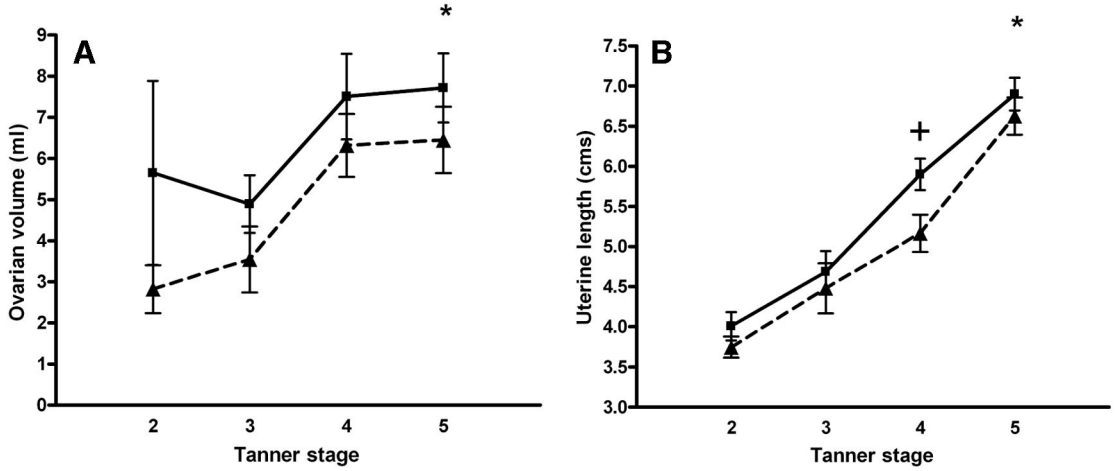

FIG. 3. Transabdominal ultrasonography in DM1 and C girls according to Tanner stage (T). *Broken line* and *triangles*, C girls; *continuous line* and *squares*, DM1 girls. Data are shown as average ± SE. A, Ovarian volume. Both ovaries were measured and the larger one is described in the figure. *, Ovarian volume in DM1 larger than in C adjusted by Tanner stage, $P < 0.05$ (ANCOVA). B, Uterine length. *, DM1 *vs.* C adjusted by Tanner stage, $P < 0.05$ (ANCOVA). +, $P < 0.05$ DM1 T4 *vs.* C T4 (Mann-Whitney *U*).

males with DM1 (34). These data suggesting that DM1 may influence ovarian function by the end of puberty are in agreement with our recent observations regarding pubertal development in girls with DM1 (33). We observed that girls with DM1 show a delay in pubertal development by the final stages of puberty, and this was associated with increasing BMI and a lack of the normal decrease in the waist to hip ratio, continuing to deteriorate 2 yr after menarche. Thus, girls with DM1 appear to show differences in pubertal development and ovarian function during late puberty, compared with C.

Ovarian volume has also been proposed as a diagnostic criteria of PCOS (28). To our knowledge, the larger ovarian volume observed in pubertal patients with DM1 has not been reported previously. This finding may represent an early sign of PCOS, as demonstrated by a longitudinal assessment of ovarian size in girls with irregular menses (35). Venturoli *et al.* (35) showed that enlarged ovaries in perimenarchal girls may persist over time and may be associated with subsequent hyperandrogenism.

We observed subtle evidence of clinical hyperandrogenism as slight hirsutism in 10% of pubertal girls with DM1. In contrast, our C girls did not show any evidence of abnormal hair growth. Moreover, the prevalence of hirsutism in these girls was greater than the reported 5% in adult Chilean women (14). Half of these girls had elevated androgen levels, but three had normal steroid concentrations, including normal FAI, suggesting that some girls with DM1 had idiopathic hirsutism. The previous study by Escobar-Morreale (2) showed a mean Ferriman-Gallway score of 11 in 17% of adult women with DM1, being less severe than in hyperandrogenic women without DM1 (36). Careful follow-up of our patients will clarify whether this slight hirsutism evolves into more severe hirsutism later in life. *In vitro* studies have shown that insulin is essential for hair follicle growth (37), and an acceleration of hair growth with insulin treatment has been reported (38).

In summary, adolescents with DM1 show increasing levels of 17OHProg after stimulation with leuprolide acetate during late puberty, associated with decreasing levels of SHBG and increasing FAI. Moreover, a higher prevalence of hirsutism during puberty was observed in girls with DM1, compared with C. These data suggest that patients with DM1 have differences in ovarian steroidogenesis, compared with normal girls during puberty. Future studies in young women should clarify whether these findings are related to the pathogenesis of hyperandrogenism later in life.

Acknowledgments

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Erratum

In the article "Metformin Administration *versus* Laparoscopic Ovarian Diathermy in Clomiphene Citrate-Resistant Women with Polycyctic Ovary Syndrome: A Prospective Parallel Randomized Double-Blind Placebo-Controlled Trial" by S. Palomba, F. Orio Jr., L. G. Nardo, A. Falbo, T. Russo, D. Corea, P. Doldo, G. Lombardi, A. Tolino, A. Colao, and F. Zullo
errors appear in one author's institutional affiliation and in data reported in Tables 2 and 3.

On the title page, the correct affiliation of Dr. Luciano Giovanni Nardo is Department of Obstetrics and Gyneacology, Wexham Park Hospital, Slough, UK.

In the Abstract, the correct data for ovulation rate in group B is 53.2%, whereas the correct data for pregnancy, abortion, and live-birth rates for group A are 21.8%, 9.3%, and 86.0%, respectively.

In *Results* , the correct data are: 197 as total number of cycles in group A; 53.2% as ovulation rate in group B, and 21.8%, 9.3%, and 86.0% as pregnancy, abortion, and live-birth rates for group A, respectively; 79.6% (43/54) as cumulative pregnancy rate for group A; 3 of 11 as patients of group A having regular ovulatory menstrual cycles at the end of the study. In the Table 2 *(page 4805)*, the correct data are 25/54 (46.3), 23/46 (50.0), 20/36 (55.6), 17/26 (65.4), 13/20 (65.0), 10/15 (66.7) for the ovulation rates of group A during the six cycles of treatment, while for the pregnancy rates of group A during the 3rd, 4th, 5th, and 6th cycle the respective correct data are 10/36 (27.8), 6/26 (23.1), 5/20 (25.0), and 4/15 (26.7); all comparisons between two groups are not significant. In the Table 3 *(page 4805),* the correct data are 108/197 (54.8), 43/197 (21.8), 4/43 (9.4), and 37/43 (86.0), respectively, for ovulation, pregnancy, abortion, and live-birth rates in group A.