

Diagnostic Criteria for Polycystic Ovary Syndrome and Ovarian Morphology in Women with Type 1 Diabetes Mellitus

Ethel Codner, Nestor Soto, Patricia Lopez, León Trejo, Alejandra Ávila, Francisca C. Eyzaguirre, Germán Íñiguez, and Fernando Cassorla

Institute of Maternal and Child Research (E.C., P.L., L.T., F.C.E., G.I., F.C.), School of Medicine, University of Chile, and Hospital San Borja Arriarán (N.S., A.A.), Santiago, Chile 836-0160

Context: The criteria for diagnosis of polycystic ovary syndrome (PCOS) have been modified and now include polycystic ovary morphology (PCOM).

Objective: The purpose of this study was to determine the frequency of PCOS and PCOM in women with type 1 diabetes mellitus (DM1).

Design: We evaluated the clinical, hormonal, and ultrasonographic characteristics in women with DM1 and compared them with a carefully matched group of normal women in a cross-sectional study.

Setting: The study was conducted at an academic research institute located within a general hospital.

Patients: All the women with DM1 attending our hospital who had experienced menarche at least 2.5 yr earlier were invited to participate and were compared with healthy women with regular menses and without a history of hyperandrogenism [controls (C)].

Results: Hirsutism was present in 28.6 and 0.0% of DM1 and C, respectively ($P < 0.001$). Biochemical hyperandrogenism was present in 23.8 and 7.9% of DM1 and C, respectively. DM1 women had higher levels of testosterone and androstenedione and larger ovarian volume and follicle number by ovary than C. PCOM was present in 54.8% of DM1 and 13.2% of C ($P < 0.001$). Oligomenorrhea was present in 19% of women with DM1. The frequency of PCOS was 40.5 and 2.6% in DM1 and C, respectively (relative risk, 15.4; 95% confidence interval, 2.2–110.2; $P < 0.0001$). The proportion of women using intensive insulin treatment was higher in those with PCOM/PCOS ($P < 0.05$). Intensive treatment was a significant factor over having PCOM/PCOS ($P < 0.05$).

Conclusions: A high frequency of hyperandrogenism, PCOM, and PCOS is observed in DM1, which appears to be associated with intensive insulin treatment.

POLYCYSTIC OVARY SYNDROME (PCOS) is frequently associated with insulin resistance and type 2 diabetes mellitus. In 2000 Escobar-Morreale *et al.* (1), using National Institutes of Health (NIH) 1990 diagnostic criteria, reported a prevalence of 18.8% for PCOS in women with diabetes mellitus type 1 (DM1). Recently the PCOS diagnostic criteria have been modified (2), so the frequency of PCOS using the new diagnostic criteria in adult women with DM1 is not known.

The new diagnostic criteria, developed by the European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine, which is known as the Rotterdam consensus criteria for the diagnosis of PCOS, includes polycystic ovary morphology (PCOM) as a key element of the diagnostic triad. PCOM is diagnosed if an increased number of follicles or ovarian volume is present. Recently we documented an elevated ovarian volume in pubertal girls with DM1 (3), but it is not known whether

adult women with DM1 have an ovarian morphology compatible with the diagnosis of PCOM.

The physiopathology of PCOS in DM1 is not clear. It has been suggested that the use of exogenous insulin to treat DM1 in these patients may contribute to the development of PCOS (1). Insulin is administered in a nonphysiological fashion, potentially stimulating the synthesis of androgens by the ovaries (4). We postulate that intensive insulin treatment may be involved in the development of PCOS/PCOM in women with DM1. The purpose of this study was to determine the frequency of PCOS and PCOM in postpubertal women with DM1 and evaluate whether these abnormalities are related to intensive insulin treatment. We evaluated the clinical, hormonal, and ultrasonographic characteristics in a group of women with DM1 and compared these results with a carefully matched group of normal women in a cross-sectional study.

Subjects and Methods

Subjects

All the women with DM1 attending the diabetes clinic of Hospital San Borja-Arriarán, Santiago, who experienced menarche at least 2.5 yr earlier, were invited to participate. This diabetes unit takes care of all the patients with DM1 in the public health system who live in central Santiago. Patients were included in this study if they had persistent insulinopenia or a C-peptide level 0.05 nmol/liter or less and were between the ages of 15 and 40 yr. We excluded from the study patients

Abbreviations: BMI, Body mass index; C, control(s); DHEAS, dehydroepiandrosterone sulfate; DM1, diabetes mellitus type 1; FG, Ferriman-Gallway; FTC, calculated free testosterone; HbA1c, hemoglobin A1c; 17OH progesterone, 17-hydroxyprogesterone; PCOM, polycystic ovary morphology; PCOS, polycystic ovary syndrome; T, testosterone.

with specific types of diabetes mellitus, type 2 diabetes mellitus, honeymoon period or diabetes duration of less than 1 yr, and presence of diabetic nephropathy.

Daily insulin dose used during the last 15 d before study was recorded and expressed as units per kilogram per day. All women, except one who was using glargine, were receiving intermediate (NPH) and soluble (either regular, lispro, or aspart) insulin. Women with DM1 were classified as having intensive treatment if they used three or more doses of insulin per day and as conservative if a fewer number of injections was used. Although we began to intensively treat patients with DM1 in the year 1999 (5), this type of treatment was not covered by the Chilean Governmental Insurance until 2005, which explains why some of the patients reported in this study received conservative treatment.

Healthy postmenarcheal women without a history of hyperandrogenism and who had regular menstrual cycles, 24–34 d in length were recruited as controls ($n = 38$). Women with DM1 and control (C) groups were matched according to chronological age and body mass index (BMI) and were excluded from the study if they were pregnant during the last 6 months; used sex steroids; or had abnormal thyroid function or prolactin levels or the presence of chronic conditions such as genetic syndromes, celiac disease, renal, liver or cardiac disease, or undernourishment.

Study protocol

A complete physical examination was performed by two of the authors (E.C. and N.S.). Hirsutism was evaluated by determining the presence of terminal hair using the modified Ferriman-Gallway (FG) score (6), and we evaluated the presence of acne. 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.

An early-morning sample of blood was obtained in both groups of women during the follicular phase (d 1–7) for measurement of testosterone (T), androstenedione, 17-hydroxyprogesterone (17OH progesterone), dehydroepiandrosterone sulfate (DHEAS), and SHBG, as previously described (3, 7). Free testosterone (FTC) was calculated as previously described (8).

Ultrasonography was performed and analyzed by a single observer (L.T.) who was blinded to the condition of the subject. Whenever possible, the exam was performed transvaginally with a 7.5-MHz transvaginal probe using Medison Sonoace 6000C equipment (Medison Co., Seoul, Korea). In a similar proportion of DM1 and C women (40.4 vs. 34.2%, respectively, $P = 0.7$), ultrasonography was carried out transabdominally using a 5-MHz abdominal probe. Ovarian volume was calculated using the simplified formula for a prolate ellipsoid (9), and the ovary with the larger volume ovary is reported. The follicle number was obtained counting the number of 2- to 9-mm follicles in the longitudinal cross-section of the ovary. The number of follicles of the ovary with the larger number of follicles is reported.

The protocol was approved by the Institutional Review Board of the San Borja Arriarán Hospital. All patients older than 18 yr signed informed consents, and for younger patients the consent was signed by their parents.

Definition of PCOS and PCOM

PCOS was defined according to the European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine Rotterdam consensus criteria for the diagnosis of PCOS (2). Clinical hyperandrogenism was diagnosed if the FG score was 8 or greater or the patient had moderate to severe acne, defined by the presence of inflammatory lesions and their extension (10). Biochemical hyperandrogenism was defined if T or FTC or androstenedione were above the 95% confidence interval for the 97.5 percentile in C women (11). Oligomenorrhea was defined if menses occurred less than nine times a year or if three cycles more than 36 d long occurred during the last year.

As mentioned, PCOM was defined using the newly described criteria from the Rotterdam consensus in which there was the presence of 12 or more follicles measuring 2–9 mm in diameter and/or an ovarian volume greater than 10 ml in one or both ovaries (2, 12).

Statistical analysis

Clinical and laboratory data are shown as mean \pm SEM. Variables were tested for normal distribution using the Kolmogorov-Smirnov test. The data that were not normally distributed (LH, FSH, and FTC only) were log transformed. Differences between women with DM1 and C for continuous variables were assessed with the Student's *t* test. Differences in proportions between the two groups were evaluated using Fisher's exact test and also reported as relative risk with its 95% confidence interval. Differences between women with DM1 without PCOS/PCOM and those with PCOS or PCOM were assessed by one-way ANOVA; if this test showed a significant difference among the three groups, it was followed by the least significant differences test for multiple comparisons. Differences in proportions among these three groups were evaluated using Pearson's χ^2 test.

The effect of intensive treatment, BMI, waist to hip ratio, insulin dose, metabolic control, and onset of diabetes before menarche over PCOS/PCOM was analyzed using simple binary logistic regression. The effect of these variables over T levels was evaluated using multiple linear regression. All statistic calculations were run on SPSS for Windows (version 10.0; SPSS, Chicago, IL) and GraphPad Prism (version 4.0 for Windows; Graph-Pad Software, San Diego, CA). $P < 0.05$ was considered statistically significant.

Results

Forty-two women with DM1 (aged 23.4 ± 1.1 yr) and 38 C women (aged 26.3 ± 1.2 yr) were studied. Their clinical characteristics are shown in Table 1. Although BMI was similar in both groups, waist to hip ratio was larger in women with DM1 than in C ($P < 0.0001$). The gynecological age was 10.8 ± 1.1 yr in women with DM1 and 13.0 ± 1.2 yr. in C ($P = 0.18$).

Hirsutism was more prevalent in the DM1 women than C [12 women with DM1 (28.6%) vs. 0 (0.0%) in C, $P < 0.001$; Fig. 1]. FG score was higher in women with DM1 than C (5.2 ± 0.6 vs. 1.1 ± 0.2 , respectively, $P < 0.001$). The range of the FG score was 0–14 and 0–5 in women with DM1 and C, respectively. Most of the C women had a score of 0–2 (Fig. 1). Seven of the hirsute women with DM1 had normal androgen levels and ultrasonographic findings. Seven women with DM1 and one C had moderate to severe acne ($P = 0.08$). Of the seven women with DM1 who had moderate to severe acne, three had PCOM and one had biochemical hyperandrogenism and oligomenorrhea.

Women with DM1 had higher levels of T, androstenedione, and 17OH progesterone but similar levels of FTC,

TABLE 1. Clinical and anthropometric characteristics in DM1 and C women, as well as metabolic control in women with DM1

	DM1	C
n	42	38
Age (yr)	23.4 ± 1.1	26.3 ± 1.2
BMI (kg/m ²)	24.5 ± 0.5	24.3 ± 0.5
Height (cm)	157.3 ± 1.0	158.8 ± 1.0
Waist-to-hip ratio	81.7 ± 0.8	77.1 ± 0.8^a
DM1 duration (yr)	11.6 ± 1.0	
HbA1c (%)	8.6 ± 0.3	
Intensive insulin treatment (%)	24 (57.1)	
Insulin dose (U/kg/d)	1.1 ± 0.1	

Data are shown as mean \pm SEM.

^a $P < 0.0001$.

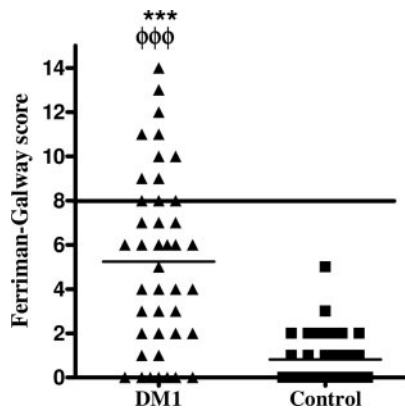


FIG. 1. FG score in women with DM1 and C. The longer horizontal line limits the upper value of normality. Small horizontal line shows the average. ***, $P < 0.001$, average FG score in women with DM1 vs. C (Student's t test). $\phi\phi\phi$, $P < 0.001$, proportion of subjects with hirsutism in women with DM1 vs. C (Fisher's exact test).

SHBG, DHEAS, FSH, LH, FSH to LH ratio, and estradiol levels (Table 2). The number of women with elevated androgen levels is shown in Fig. 2.

PCOM and ultrasonographic findings (Table 3)

Women with DM1 had a larger ovarian volume ($P < 0.01$) and follicle number ($P < 0.01$). An elevated number of follicles was present in 15 and four women with DM1 and C, respectively ($P < 0.01$), and an abnormal ovarian volume was observed in 17 women with DM1 and four controls ($P < 0.01$). PCOM morphology was found in 23 women with DM1 (54.8%) and five C (13.2%) (relative risk 4.1; 95% confidence interval 1.8–9.9; $P < 0.001$).

PCOS (Table 4)

Hyperandrogenism, defined as the presence of clinical or biochemical hyperandrogenism, was present in 21 (50%) and four (10.5%) of the women with DM1 and C, respectively (relative risk 4.8; 95% confidence interval 1.8–12.6; $P < 0.001$).

TABLE 2. Basal steroids and gonadotropins in women with DM1 and C

	DM1 (n = 42)	C (n = 38)
T (ng/dl)	61.5 ± 36.0	49.5 ± 2.0 ^a
Androstenedione (ng/ml)	1.8 ± 0.1	1.4 ± 0.0 ^a
DHEAS (ng/ml)	1336.2 ± 92.5	1316.7 ± 78.9
FTC (ng/dl)	1.0 ± 0.1	0.8 ± 0.1
SHBG (μg/dl)	428.4 ± 31.5	423.8 ± 32.8
Estradiol (pg/ml)	64.2 ± 5.1	71.3 ± 4.8
FSH (IU/liter)	5.5 ± 0.4	5.9 ± 0.7
LH (IU/liter)	6.2 ± 1.0	8.1 ± 3.1
LH/FSH ratio	1.2 ± 0.2	1.1 ± 0.1
17OH progesterone (ng/ml)	1.7 ± 0.1	1.5 ± 0.1 ^b

Data are shown as mean ± SEM. To convert units to SI: T, 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; FTC, nanograms per deciliter × 34.67 = picomoles per liter; SHBG, micrograms per deciliter × 0.1111 = nanomoles per liter; estradiol, picograms per milliliter × 3.67 = picomoles per liter; 17OH progesterone, nanograms per milliliter × 3.03 = nanomoles per liter.

^a $P < 0.01$.

^b $P < 0.05$.

Clinical hyperandrogenism was present in 16 women with DM1 and one C ($P < 0.001$). Biochemical hyperandrogenism was present in 10 and three women with DM1 and C, respectively ($P = 0.07$). Five women with DM1 and no C had clinical and biochemical hyperandrogenism ($P = 0.055$). Five DM1 and three C had biochemical hyperandrogenism without clinical signs. Eight women with DM1 but no C exhibited menstrual irregularities ($P < 0.01$).

The frequency of PCOS in the women with DM1 and C was 40.5 and 2.6%, respectively (relative risk 15.4; 95% confidence interval 2.2–110.2; $P < 0.0001$). Two women with DM1 and no C showed the three criteria necessary for diagnosis of PCOS, the former having simultaneously elevated T levels, oligomenorrhea, and PCOM, without clinical hyperandrogenism. Fifteen DM1 and one C exhibited two of the three criteria simultaneously. Two women with DM1 exhibited oligomenorrhea and PCOM without hyperandrogenism. Three DM1 showed oligomenorrhea and hyperandrogenism (two of them having simultaneously clinical and biochemical hyperandrogenism, and the remaining one had only biochemical hyperandrogenism). Ten women with DM1 had hyperandrogenism and PCOM, three of them having simultaneously clinical and biochemical hyperandrogenism and PCOM, five having clinical hyperandrogenism and PCOM, and the remaining two had biochemical hyperandrogenism and PCOM. One C woman exhibited biochemical hyperandrogenism and PCOM.

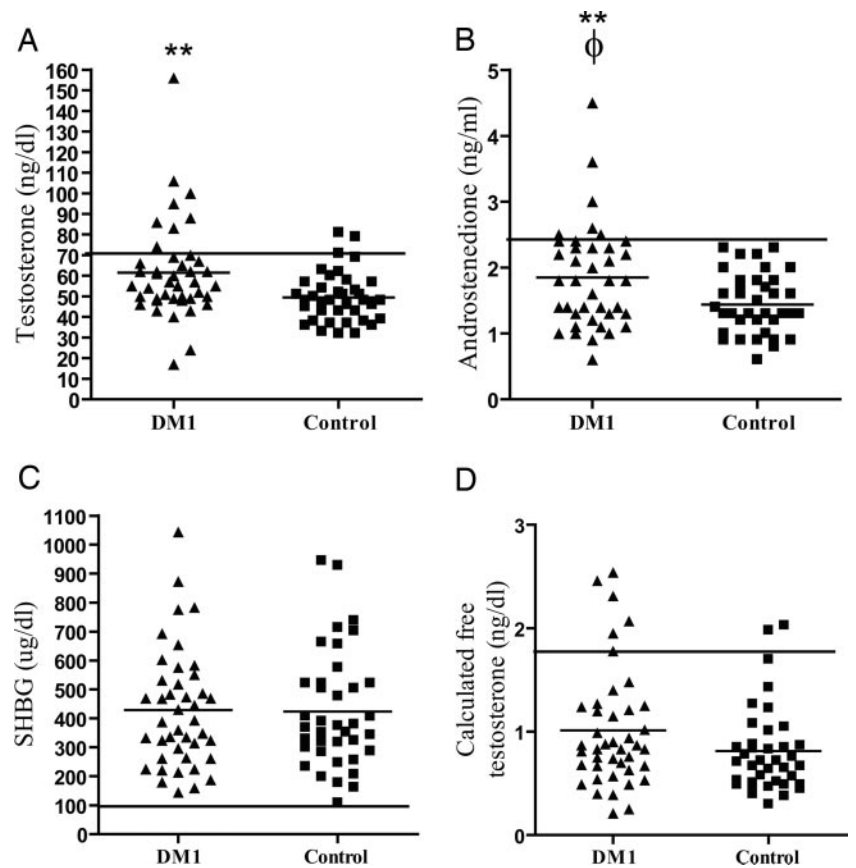
Clinical and laboratory findings in women with DM1 with and without PCOS/PCOM (Table 5)

Women with DM1 were classified into three groups, those without PCOS or PCOM, those with PCOM only, and those who fulfilled criteria for PCOS. The proportion of women using intensive treatment in the PCOS or PCOM group was higher than in the women with normal findings ($P < 0.05$, Pearson's χ^2). Moreover, 75 and 33% of the women using intensive and conservative treatment, respectively, had either PCOM or PCOS ($P = 0.059$, intensive vs. conservative treatment).

Women with DM1 and PCOS had higher FTC and DHEAS levels than the other two groups; however, SHBG levels were similar to the women with DM1 without PCOS/PCOM. T levels almost reached a significant difference among the three groups (ANOVA, $P = 0.07$). Women with PCOM had higher SHBG than women with normal findings or women with DM1 and PCOS. Ovarian volume and follicle number were similar in women with PCOS and PCOM but higher than women with DM1 with neither.

Binary logistic regression showed intensive treatment to be a significant factor over exhibiting PCOS or PCOM (beta = 1.32, SE = 0.669, $P = 0.048$). BMI, waist to hip ratio, insulin dose, hemoglobin A1c (HbA1c), and onset before menarche did not show a significant effect over the presence of PCOS or PCOM. In addition, multiple regression analysis determined that these factors, including intensive treatment, did not have a significant effect over T or androstenedione levels.

FIG. 2. T, androstenedione, SHBG, and FTC levels in women with DM1 and C. The longer horizontal lines limit the upper value of normality, as obtained from the C women (see *Subjects and Methods*). In the case of SHBG (panel C), the longer horizontal line limits the lower value of normality. Small horizontal line shows the average. A, Testosterone. B, Androstenedione. C, SHBG. D, FTC levels. **, $P < 0.01$, comparison of the average value in women with DM1 vs. C (Student's t test); ϕ , $P < 0.05$, proportion of subjects with abnormal results in women with DM1 vs. C (Fisher's exact test). To convert units to SI: T, nanograms per deciliter $\times 0.0347$ = nanomoles per liter; androstenedione, nanograms per milliliter $\times 3.49$ = nanomoles per liter; FTC, nanograms per deciliter $\times 34.67$ = picomoles per liter; SHBG, (micrograms per deciliter) $\times 0.1111$ = nanomoles per liter.



Discussion

We report a clinical, hormonal, and ultrasonographic study of hyperandrogenism in adult women with DM1, using the new Rotterdam consensus, compared with a control group carefully matched by age and BMI. Our study confirms the previous findings by Escobar-Morreale *et al.* (1) of a high frequency of hyperandrogenic disorders in women with DM1 in a Spanish population, showing that they are also present in a population with different ethnicity. In addition, our data expand previous findings by showing that PCOM is even more frequent than PCOS in these patients. In addition, our results suggest that PCOS/PCOM may be related to intensive insulin treatment.

Hyperandrogenism was present in 50% of our patients and 40% of patients studied in the Spanish series (1). Moreover, our patients with DM1 had higher levels of serum T and

TABLE 3. Gynecological ultrasonography in women with DM1 and C

	DM1 (n = 42)	Control (n = 38)
Ovarian volume (ml)	9.3 \pm 0.6	7.1 \pm 0.4 ^a
Follicle no.	10.0 \pm 0.8	6.9 \pm 0.5 ^a
PCOM (%)	23 (54.8)	5 (13.2) ^b
Follicle no. \geq 12 (%)	15 (35.7)	4 (10.5) ^a
Ovarian volume > 10 ml (%)	17 (42.5)	4 (10.5) ^a

The ovarian volume and the follicle number were obtained from the ovary with the largest volume and follicle number, respectively. Data are shown as mean \pm SEM.

^a $P < 0.01$.

^b $P < 0.001$.

androstenedione, compared with controls. These findings suggest that the clinician taking care of women with DM1 should be aware of the high frequency of hyperandrogenism in these women, especially because 12% of our patients exhibited biochemical hyperandrogenism without any clinical signs.

The results in our series also confirm the high frequency of hirsutism present in women with DM1, which was 28.6% in our patients and 30.6% in the Spanish series. However, the mean score and range of the hirsutism found by us is lower than in the Spanish patients, which may be related to ethnicity (13).

Our study showed that 40.5% of the women with DM1 had PCOS according to the Rotterdam criteria, which is higher than the previously reported frequency of 18.8%, using 1990 NIH diagnostic criteria. This difference is due to the high rate of PCOM observed in our series, which was not included as a diagnostic criterion in the NIH definition. In our study, if we had used NIH criteria, the prevalence of PCOS would have been 11.9% (Table 4). The Spanish group who performed the evaluation of the prevalence of PCOS in women with DM1 also showed a prevalence of 6.5% in healthy women (14), which is lower than the 18.8% they observed in women with diabetes mellitus. A limitation of our study is the lack of an established prevalence for PCOS in patients without DM1 in Chile. However, diverse studies performed around the world have shown a prevalence of 5–10% of PCOS in premenopausal women without DM1 (14–17).

PCOS in women with DM1 has several differences from

TABLE 4. PCOS in women with DM1 and C

	DM1 (n = 42)	C (n = 38)	P	Relative risk (95% confidence interval)
PCOS (%)	17 (40.5)	1 (2.6)	<0.0001	15.4 (2.2–110.2)
Oligomenorrhea + PCOM + hyperandrogenism	2 (4.8)	0 (0)	0.50	
Oligomenorrhea + PCOM	2 (4.8)	0 (0)	0.50	
Oligomenorrhea + hyperandrogenism	3 (7.1)	0 (0)	0.20	
Hyperandrogenism + PCOM	10 (23.8)	1 (2.6)	0.0078	9.0 (1.2–67.4)
PCOM (%)	23 (54.8)	5 (13.2)	0.00013	4.1 (1.8–9.9)
Hyperandrogenism	21 (50)	4 (10.5)	0.0002	4.8 (1.8–12.6)
Clinical hyperandrogenism	16 (38.1)	1 (2.6)	<0.0001	14.5 (2.0–104.1)
Hirsutism with biochemical hyperandrogenism	5 (11.9)	0 (0)	0.056	
Hirsutism without biochemical hyperandrogenism	7 (16.6)	0 (0)	0.0125	
Acne with biochemical hyperandrogenism	1 (2.4)	0 (0)	1.00	
Acne without biochemical hyperandrogenism	6 (14.3)	1 (2.6)	0.11	
Biochemical hyperandrogenism	10 (23.8)	3 (7.9)	0.07	3.0 (0.9–10.2)
Oligomenorrhea	8 (19.0)	0 (0)	0.0058	

Hyperandrogenism was defined according to the Rotterdam criteria as the presence of clinical or biochemical hyperandrogenism (*Subjects and Methods*). Data in parentheses represent percentage or 95% confidence interval.

what is observed in women without DM1. Clinically the severity of the hirsutism is lower than in nondiabetic hyperandrogenic women. Roldan *et al.* (18) compared 14 women with DM1 and hyperandrogenism with a group of nondiabetic hyperandrogenic women and showed that the FG score is lower in the former group. The average score of hirsutism observed in our patients is even lower than that published by Escobar-Morreale *et al.* (1). This lower degree of hirsutism may explain the reason why this sign may be frequently overseen by the clinician taking care of diabetic women.

The biochemical findings of hyperandrogenism in women with DM1 also show differences to what is observed in women without DM1. We did not observe a decreased level of SHBG in the whole group of DM1 or in the PCOS and DM1 subgroup, which may explain why total T was the more sensitive index of hyperandrogenism in this group (Fig. 2).

This is different from what is observed in women without DM1, in whom the most frequent biochemical element of hyperandrogenism is an increased level of free androgens, such as free T or free androgen index (2, 19). The different behavior in SHBG levels in women with DM1 may be related to the fact that insulin concentrations at the portal vein are the main regulators of SHBG (20), whereas in these women the hormone is administered sc.

Another difference in the laboratory findings in hyperandrogenic women with DM1 is the lack of increase of LH and LH to FSH ratio (Table 5), which is frequently observed in women with PCOS and without DM1 (21). These findings are in agreement with the ovarian origin of hyperandrogenism in women with DM1. Virdis *et al.* (22) were the first to suggest the presence of functional ovarian hyperandrogenism in women with DM1 and oligomenorrhea, and the ovarian source of androgens was confirmed by Roldan *et al.* (18) by

TABLE 5. Clinical and biochemical characteristics of the women with DM1 with and without PCOS or PCOM

	DM1 without PCOS or PCOM (n = 16)	DM1 with PCOM (n = 9)	DM1 with PCOS (n = 17)
Age (yr)	26.3 ± 2.1	20.6 ± 2.3	22.2 ± 1.4
Gynecological age (yr)	13.5 ± 2.1	7.7 ± 2.2	10.1 ± 1.3
Insulin dose (U/kg/d)	1.1 ± 0.1	1.0 ± 0.1	1.2 ± 0.1
Intensive insulin treatment (%)	6 (37.5)	8 (88.9)	8 (47.1) ^c
HbA1c (%)	8.6 ± 0.5	9.1 ± 0.5	8.2 ± 0.4
Waist-to-hip ratio	81.9 ± 1.2	81.2 ± 1.8	81.6 ± 1.7
T (ng/dl)	54.6 ± 7.5	55.0 ± 2.8	71.4 ± 4.5
F _{125C} (ng/dl)	0.9 ± 0.1	0.7 ± 0.1	1.3 ± 0.1 ^c
SHBG (μg/dl)	402.0 ± 36.2	596.3 ± 87.3 ^b	365.1 ± 42.5
17OH progesterone (ng/ml)	1.5 ± 0.2	1.6 ± 0.2	0.2 ± 2.4
DHEAS (ng/ml)	1074.0 ± 139.6	1229.2 ± 95.0	1639.6 ± 161.4 ^d
Androstenedione (ng/ml)	1.6 ± 0.2	1.8 ± 0.1	2.1 ± 0.2
LH/FSH ratio	1.4 ± 0.3	1.0 ± 0.3	1.2 ± 0.2
Ovarian volume (ml)	6.3 ± 0.6 ^e	10.8 ± 0.9	11.3 ± 0.7
Follicle no. by ovary (n)	6.1 ± 0.5 ^f	11.8 ± 1.6	12.3 ± 1.3

To convert units to SI: T, 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 progesterone, nanograms per milliliter × 3.03 = nanomoles per liter; F_{125C}, nanograms per deciliter × 34.67 = picomoles per liter; SHBG, micrograms per deciliter × 0.1111 = nanomoles per liter.

^a $P < 0.05$ for difference in proportion between the three groups (Pearson's χ^2).

^b $P < 0.01$ DM1 with PCOM vs. DM1 with PCOS and $P < 0.05$ DM1 with PCOM vs. DM1 without PCOS/PCOM.

^c $P < 0.05$ DM1 with PCOS vs. the other two groups.

^d $P < 0.01$ DM1 with PCOS vs. DM1 without PCOS or PCOM.

^e $P < 0.001$ DM1 without PCOS/PCOS vs. DM1 with PCOM, and $P < 0.0001$ vs. DM1 with PCOS.

^f $P < 0.01$ DM1 without PCOS/PCOS vs. DM1 with PCOM, and $P < 0.001$ vs. DM1 with PCOS.

showing a normal steroid response to ACTH in women with hyperandrogenism and DM1. In addition, recently we evaluated ovarian function in pubertal girls with DM1 using the leuprolide test, a GnRH analog, which is the best way to identify the ovary as the source of the hyperandrogenism (23, 24), and showed that during the later stages of puberty, there are elements that suggest the onset of functional ovarian hyperandrogenism (3).

Clinical and laboratory findings of hyperandrogenism in women with DM1 may have their onset during puberty but continue to progress afterward. Our data show that the hirsutism, evaluated with the FG score, is higher in adult women than in pubertal girls with DM1 (3). Similarly, our study also shows that women with DM1 have higher waist to hip ratio than control women, confirming our previous finding of a lack of decrease of this ratio during puberty (25).

PCOM/PCOS was associated with intensive insulin treatment, which was the only factor related to this variable. Total daily insulin dose, HbA1c levels, or premenarcheal onset did not show a significant effect over the development of PCOM/PCOS. This would represent a novel potential adverse effect of intensive insulin treatment, which has not been previously reported. The series of Escobar-Morreale *et al.* (1) did not find this association, but this difference may be due to the fact that most of these patients (88%) were already using intensive treatment.

It has been suggested that the use of exogenous insulin to treat DM1 may contribute to the development of PCOS (1). Insulin is administered in a nonphysiological fashion because it is injected sc and is absorbed into the systemic circulation (26). Multiple insulin doses theoretically could lead to significant hyperinsulinemia in the ovary. Hyperinsulinemia stimulates the development of antral follicles, increasing the sensitivity of granulosa cells to FSH, thus increasing the numbers of follicles and ovarian volume (27). In addition, *in vitro* studies have shown that insulin acts synergically with LH to stimulate the synthesis of T by ovarian thecal cells (4). An additional pathogenic mechanism might be the exacerbated insulin resistance observed in patients with DM1 (28), which has been proposed to play a role in the pathogenesis of PCOS (29). Other pathogenic mechanisms observed in PCOS such as increased fat mass and abnormalities in the GH/IGF-I axis may play a role because they are also present in DM1 (30, 31).

The main finding of our study is the high rate of PCOM in women with DM1. Studies in healthy and ovulatory women with PCOM have shown that this group may represent the mildest form of ovarian hyperandrogenism because it is associated with greater androgen levels and insulin resistance than in women with normal morphology (32) and with an abnormal response to a GnRH analog test (33). In addition, healthy women with PCOM have a higher prevalence of abnormal metabolic and cardiovascular risk parameters (34). In our study, women with PCOM exhibited higher SHBG levels, but no other endocrine abnormality was documented.

Our data suggest that factors associated with cardiovascular risk and insulin resistance such as increased androgen levels (35–38) or waist to hip ratio (39) are also present in women with DM1. Moreover, higher androgen levels in

women with DM1 have been associated with microalbuminuria (40).

In conclusion, we have shown a high prevalence of PCOM and PCOS in a group of women with DM1 with different ethnicity than the original report from Spain, suggesting that hirsutism and hyperandrogenism should be carefully investigated in postmenarcheal women with DM1, especially in those with intensive treatment. Future studies should evaluate the consequences of PCOM in women with DM1.

Acknowledgments

We are grateful to all the women who participated; Dr. Dennis Mook-Kanamori and Dr. Marcela Candia for initial help organizing the study; Dr. Ariel Fuentes (Institute of Maternal and Child Research, University of Chile) for advice on statistical analysis; and Ms. Leonor Varela for the excellent technical help with the ultrasonographies.

Received January 18, 2006. Accepted March 22, 2006.

Address all correspondence and requests for reprints to: Ethel Codner, M.D., Institute of Maternal and Child Research, School of Medicine, University of Chile, Casilla 226-3, Santiago, Chile. E-mail: ecodner@med.uchile.cl.

This work was supported in part by Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT) Grant 1050452 (to E.C.).

Disclosure statement: F.C. has received lecturing fees from Pfizer and Novo Nordisk. A.A. has received consulting fees from Pfizer. The remaining authors have no disclosure to report.

References

- Escobar-Morreale HF, Roldan B, Barrio R, Alonso M, Sancho J, de la Calle H, Garcia-Robles R 2000 High prevalence of the polycystic ovary syndrome and hirsutism in women with type 1 diabetes mellitus. *J Clin Endocrinol Metab* 85:4182–4187
- The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004 Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* 81:19–25
- Codner E, Mook-Kanamori D, Bazaes RA, Unanue N, Sovino H, Ugarte F, Avila A, Iniguez G, Cassorla F 2005 Ovarian function during puberty in girls with type 1 diabetes mellitus: response to leuprolide. *J Clin Endocrinol Metab* 90:3939–3945
- Cara JF, Rosenfield RL 1988 Insulin-like growth factor I and insulin potentiate luteinizing hormone-induced androgen synthesis by rat ovarian thecal-interstitial cells. *Endocrinology* 123:733–739
- Codner E, Mericq V, Garcia H, Lopez C, Caceres J, Gaete X, Avila A 2003 Results of a multidisciplinary and intensified treatment program for type 1 diabetes mellitus in a Chilean public hospital. *Rev Med Chil* 131:857–864
- Hatch R, Rosenfield RL, Kim MH, Tredway D 1981 Hirsutism: implications, etiology, and management. *Am J Obstet Gynecol* 140:815–830
- Codner E, Okuma C, Iniguez G, Boric MA, Avila A, Johnson MC, Cassorla FG 2004 Molecular study of the 3 β -hydroxysteroid dehydrogenase gene type II in patients with hypospadias. *J Clin Endocrinol Metab* 89:957–964
- Vermeulen A, Verdonck L, Kaufman JM 1999 A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab* 84:3666–3672
- Swanson M, Sauerbrei EE, Cooperberg PL 1981 Medical implications of ultrasonically detected polycystic ovaries. *J Clin Ultrasound* 9:219–222
- Witkowski JA, Parish LC 2004 The assessment of acne: an evaluation of grading and lesion counting in the measurement of acne. *Clin Dermatol* 22:394–397
- Glintborg D, Hermann AP, Brusgaard K, Hangaard J, Hagen C, Andersen M 2005 Significantly higher adrenocorticotropin-stimulated cortisol and 17-hydroxyprogesterone levels in 337 consecutive, premenopausal, Caucasian, hirsute patients compared with healthy controls. *J Clin Endocrinol Metab* 90:1347–1353
- Balen AH, Laven JS, Tan SL, Dewailly D 2003 Ultrasound assessment of the polycystic ovary: international consensus definitions. *Hum Reprod Update* 9:505–514
- Tellez R, Frenkel J 1995 Clinical evaluation of body hair in healthy women. *Rev Med Chil* 123:1349–1354
- Asuncion M, Calvo RM, San Millan JL, Sancho J, Avila S, Escobar-Morreale HF 2000 A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. *J Clin Endocrinol Metab* 85:2434–2438
- Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO 2004 The

- prevalence and features of the polycystic ovary syndrome in an unselected population. *J Clin Endocrinol Metab* 89:2745–2749
16. **Diamanti-Kandarakis E, Kouli CR, Bergiele AT, Filandra FA, Tsianateli TC, Spina GG, Zapanti ED, Bartzis MI** 1999 A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile. *J Clin Endocrinol Metab* 84:4006–4011
 17. **Michelmores KF, Balen AH, Dunger DB, Vessey MP** 1999 Polycystic ovaries and associated clinical and biochemical features in young women. *Clin Endocrinol (Oxf)* 51:779–786
 18. **Roldan B, Escobar-Morreale HF, Barrio R, de La Calle H, Alonso M, Garcia-Robles R, Sancho J** 2001 Identification of the source of androgen excess in hyperandrogenic type 1 diabetic patients. *Diabetes Care* 24:1297–1299
 19. **Imani B, Eijkemans MJ, de Jong FH, Payne NN, Bouchard P, Giudice LC, Fauser BC** 2000 Free androgen index and leptin are the most prominent endocrine predictors of ovarian response during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrhic infertility. *J Clin Endocrinol Metab* 85:676–682
 20. **Yki-Jarvinen H, Makimattila S, Utriainen T, Rutanen EM** 1995 Portal insulin concentrations rather than insulin sensitivity regulate serum sex hormone-binding globulin and insulin-like growth factor binding protein 1 *in vivo*. *J Clin Endocrinol Metab* 80:3227–3232
 21. **Fauser BC, Pache TD, Lamberts SW, Hop WC, de Jong FH, Dahl KD** 1991 Serum bioactive and immunoreactive luteinizing hormone and follicle-stimulating hormone levels in women with cycle abnormalities, with or without polycystic ovarian disease. *J Clin Endocrinol Metab* 73:811–817
 22. **Virdis R, Zampolli M, Street ME, Vanelli M, Potau N, Terzi C, Ghizzoni L, Ibanez L** 1997 Ovarian 17 α -hydroxyprogesterone responses to GnRH analog testing in oligomenorrhic insulin-dependent diabetic adolescents. *Eur J Endocrinol* 136:624–629
 23. **Barnes RB, Rosenfield RL, Burstein S, Ehrmann DA** 1989 Pituitary-ovarian responses to nafarelin testing in the polycystic ovary syndrome. *N Engl J Med* 320:559–565
 24. **Ehrmann DA, Rosenfield RL, Barnes RB, Brigell DF, Sheikh Z** 1992 Detection of functional ovarian hyperandrogenism in women with androgen excess. *N Engl J Med* 327:157–162
 25. **Codner E, Barrera A, Mook-Kanamori D, Bazaes RA, Unanue N, Gaete X, Avila A, Ugarte F, Torrealba I, Perez V, Panteon E, Cassorla F** 2004 Ponderal gain, waist-to-hip ratio, and pubertal development in girls with type 1 diabetes mellitus. *Pediatr Diabetes* 5:182–189
 26. **Bolli GB** 2001 Physiological insulin replacement in type 1 diabetes mellitus. *Exp Clin Endocrinol Diabetes* 109(Suppl 2):S317–S332
 27. **Fulghesu AM, Villa P, Pavone V, Guido M, Apa R, Caruso A, Lanzone A, Rossodivita A, Mancuso S** 1997 The impact of insulin secretion on the ovarian response to exogenous gonadotropins in polycystic ovary syndrome. *J Clin Endocrinol Metab* 82:644–648
 28. **Amiel SA, Sherwin RS, Simonson DC, Lauritano AA, Tamborlane WV** 1986 Impaired insulin action in puberty. A contributing factor to poor glycemic control in adolescents with diabetes. *N Engl J Med* 315:215–219
 29. **Dunaif A** 1997 Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. *Endocr Rev* 18:774–800
 30. **Ahmed ML, Ong KK, Watts AP, Morrell DJ, Preece MA, Dunger DB** 2001 Elevated leptin levels are associated with excess gains in fat mass in girls, but not boys, with type 1 diabetes: longitudinal study during adolescence. *J Clin Endocrinol Metab* 86:1188–1193
 31. **Bereket A, Lang CH, Wilson TA** 1999 Alterations in the growth hormone-insulin-like growth factor axis in insulin dependent diabetes mellitus. *Horm Metab Res* 31:172–181
 32. **Adams JM, Taylor AE, Crowley Jr WF, Hall JE** 2004 Polycystic ovarian morphology with regular ovulatory cycles: insights into the pathophysiology of polycystic ovarian syndrome. *J Clin Endocrinol Metab* 89:4343–4350
 33. **Chang PL, Lindheim SR, Lowre C, Ferin M, Gonzalez F, Berglund L, Carmina E, Sauer MV, Lobo RA** 2000 Normal ovulatory women with polycystic ovaries have hyperandrogenic pituitary-ovarian responses to gonadotropin-releasing hormone-agonist testing. *J Clin Endocrinol Metab* 85:995–1000
 34. **Carmina E, Chu MC, Longo RA, Rini GB, Lobo RA** 2005 Phenotypic variation in hyperandrogenic women influences the findings of abnormal metabolic and cardiovascular risk parameters. *J Clin Endocrinol Metab* 90:2545–2549
 35. **Huppert J, Chiodi M, Hillard PJ** 2004 Clinical and metabolic findings in adolescent females with hyperandrogenism. *J Pediatr Adolesc Gynecol* 17:103–108
 36. **Golden SH, Ding J, Szklo M, Schmidt MI, Duncan BB, Dobs A** 2004 Glucose and insulin components of the metabolic syndrome are associated with hyperandrogenism in postmenopausal women: the atherosclerosis risk in communities study. *Am J Epidemiol* 160:540–548
 37. **Santoro N, Torrens J, Crawford S, Allsworth JE, Finkelstein JS, Gold EB, Korenman S, Lasley WL, Luborsky JL, McConnell D, Sowers MF, Weiss G** 2005 Correlates of circulating androgens in mid-life women: the study of women's health across the nation. *J Clin Endocrinol Metab* 90:4836–4845
 38. **Sutton-Tyrrell K, Wildman RP, Matthews KA, Chae C, Lasley BL, Brockwell S, Pasternak RC, Lloyd-Jones D, Sowers MF, Torrens JI** 2005 Sex-hormone-binding globulin and the free androgen index are related to cardiovascular risk factors in multiethnic premenopausal and perimenopausal women enrolled in the Study of Women Across the Nation (SWAN). *Circulation* 111:1242–1249
 39. **Yusuf S, Hawken S, Ounpuu S, Bautista L, Franzosi MG, Commerford P, Lang CC, Rumboldt Z, Onen CL, Lisheng L, Tanomsup S, Wangai Jr P, Razak F, Sharma AM, Anand SS** 2005 Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case-control study. *Lancet* 366:1640–1649
 40. **Amin R, Schultz C, Ong K, Frystyk J, Dalton RN, Perry L, Orskov H, Dunger DB** 2003 Low IGF-I and elevated testosterone during puberty in subjects with type 1 diabetes developing microalbuminuria in comparison to normoalbuminuric control subjects: the Oxford Regional Prospective Study. *Diabetes Care* 26:1456–1461