

Serum uric acid concentration as non-classic cardiovascular risk factor in women with polycystic ovary syndrome: effect of treatment with ethinyl-estradiol plus cyproterone acetate versus metformin

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BACKGROUND: Serum uric acid levels have emerged as a cardiovascular risk factor, and interventions aimed to decrease its level have been related with an improvement in clinical and non-clinical cardiovascular outcomes. **METHODS:** Serum uric acid levels were measured in 40 polycystic ovary syndrome (PCOS) patients and 40 non-hyperandrogenic women matched for BMI and grade of obesity, and were followed-up in 34 PCOS patients who were randomized to an oral contraceptive containing 35 mg ethinyl-estradiol plus 2 mg cyproterone acetate (Diane³⁵ Diario) or metformin (850 mg twice daily) for 24 weeks. **RESULTS:** There were no statistically significant differences in uric acid levels between PCOS and non-hyperandrogenic control women. Considering all PCOS and non-hyperandrogenic control women as a whole, obese women showed higher uric acid concentrations than lean and overweight women, and the main determinant of serum uric acid level was the BMI. In PCOS women, Diane³⁵ Diario treatment was related with a decrease in uric acid levels ($P = 0.018$), whereas no changes were observed with metformin. **CONCLUSIONS:** Obesity is the main determinant of serum uric acid concentrations in PCOS patients, yet amelioration of androgen excess with an antiandrogenic contraceptive pill results in a significant decrease in these levels, an effect that is not observed with metformin. ClinicalTrials.gov NLM Identifier: NCT00428311.

Keywords: obesity; metformin; insulin resistance; hyperandrogenemia; clinical trial

Introduction

Uric acid exerts proinflammatory, prooxidant and proliferative actions at the endothelial cell level that may increase cardiovascular risk (Hayden and Tyagi, 2004; Kanellis and Kang, 2005). The increase in serum uric acid concentrations is related to cardiovascular events in high-risk subjects (Johnson *et al.*, 2003; Baker *et al.*, 2005), yet this relationship is less established in the general population (Brand *et al.*, 1985; Culleton *et al.*, 1999; Liese *et al.*, 1999; Fang and Alderman, 2000; Moriarity *et al.*, 2000) and the possible roles of uric acid as a causal agent or as a mere marker of cardiovascular risk are debated at present (Alderman, 2002).

In conceptual agreement with a causal role for uric acid on cardiovascular events, the decrease in the serum uric acid concentrations of high-risk patients following blockade of xanthine-oxydase with allopurinol resulted in an improvement

of endothelial function (Farquharson *et al.*, 2002; Mercurio *et al.*, 2004) and a reduction in cardiovascular events (Struthers *et al.*, 2002; Weimert *et al.*, 2003). Furthermore, the decrease in uric acid concentrations observed during treatment with the angiotensin-II receptor blocker losartan (Hoiegggen *et al.*, 2004) and with atorvastatin (Athyros *et al.*, 2004) has been suggested to play a role in the decreased rate of cardiovascular events associated with the use of both drugs.

Classic and non-classic cardiovascular risk markers cluster in women with the polycystic ovary syndrome (PCOS) (Ehrmann *et al.*, 1999; Legro *et al.*, 2001; Luque-Ramírez *et al.*, 2007a), the commonest endocrine-metabolic disorder in premenopausal women (Diamanti-Kandarakis *et al.*, 1999; Asuncion *et al.*, 2000; Azziz *et al.*, 2004). PCOS is a mainly hyperandrogenic disorder that is characterized by clinical and/or biochemical hyperandrogenism together with

disordered ovarian function and/or morphology (Azziz *et al.*, 2006), and is frequently associated with abdominal adiposity, obesity, insulin resistance, chronic low-grade inflammation and increased oxidative stress (Escobar-Morreale and San Millan, 2007). Although increased uric acid may influence some of these associations, the studies available at present regarding serum uric acid levels in PCOS patients are scarce and led to controversial results (Anttila *et al.*, 1996; Quiñonez Zarza *et al.*, 2000; Yarali *et al.*, 2001).

Oral contraceptives have been the mainstay of pharmacological treatment of PCOS for decades (Ehrmann, 2005). However, in non-hyperandrogenic women oral contraceptives might adversely influence insulin resistance and glucose tolerance, raising concern about a possible worsening of the already unfavorable metabolic cardiovascular risk profile of PCOS patients and favoring the use of the metabolically safer insulin sensitizer drugs (Diamanti-Kandarakis *et al.*, 2003).

We have studied here the serum uric acid concentrations of well-defined populations of PCOS patients and non-hyperandrogenic control women, as well as the effects that treatment of PCOS with an antiandrogenic oral contraceptive, as compared with those of the insulin sensitizer metformin, exert on this non-classic cardiovascular risk marker.

Materials and Methods

The present study derives from a more ample project aiming to study classic and non-classic cardiovascular risk factors in PCOS patients, and the impact of an antiandrogenic oral contraceptive, as compared with those of the insulin sensitizer metformin, on these risk factors. Forty unselected hyperandrogenic PCOS patients (age 25.6 ± 6.0 year, range 15–42 year; BMI 29.4 ± 6.3 , range 18.8–47.5 kg/m²) were recruited. Eleven of these patients were lean (BMI <25 kg/m²), 13 were overweight (BMI 25.0–29.9 kg/m²) and 16 were obese (BMI ≥ 30.0 kg/m²). The diagnosis of PCOS was based on the presence of clinical and/or biochemical hyperandrogenism, oligo-ovulation and exclusion of secondary etiologies, and therefore fulfilled all the current definitions of PCOS (Zawadzki and Dunaif, 1992; The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group, 2004; Azziz *et al.*, 2006). Of note, ovarian morphology was not evaluated in these patients, and therefore the results derived from the present study should not be extrapolated to PCOS patients in whom the finding of polycystic ovarian morphology was the hallmark for this diagnosis.

Hirsutism was defined by a modified Ferriman-Gallwey score >7 (Hatch *et al.*, 1981). Oligomenorrhea [more than 6 cycles longer than 36 days in the previous year (Goodman, 2007)], amenorrhea [absence of menstruation for 3 consecutive months (Goodman, 2007)] and luteal phase progesterone measurements <12.7 nmol/l in women with regular menstrual cycles were considered indicative of oligo-ovulation. We also excluded hyperprolactinemia by confirming serum prolactin levels <24 µg/l, thyroid dysfunction when serum thyrotropin levels were within the normal range, congenital adrenal hyperplasia by confirming cosyntropin-stimulated serum 17-hydroxyprogesterone levels <30 nmol/l and virilizing tumors in all the patients (Azziz *et al.*, 2006).

Cross-sectional study

The PCOS patients were compared with a control group composed of non-hyperandrogenic women who had regular menstrual cycles every 26–34 days (age 25.6 ± 6.0 year, range 13–38 year; BMI 29.4 ± 6.9 ,

range 19.8–49.2 kg/m²). This control group included 11 healthy female volunteers and 29 patients who did not have any known metabolic comorbidity and reported to the clinical practice of the authors solely for treatment of weight excess. The controls were selected in order to be similar in terms of age and BMI with the patients, and therefore 11 controls were lean, 13 were overweight and 16 were obese. None of the controls had signs or symptoms of hyperandrogenism, menstrual dysfunction or history of infertility.

None of the patients and controls had either a personal history of hypertension, alcohol abuse, disorders of glucose tolerance, hyperuricemia, cardiovascular events, sleep apnea or had received treatment with oral contraceptives, antiandrogens, insulin sensitizers or drugs that might interfere with uric acid levels for the previous 6 months.

Clinical trial

Thirty-four of the 40 PCOS patients included in the casecontrol study agreed to participate in a randomized controlled clinical trial addressing the effects of treatment with an antiandrogenic oral contraceptive compared with the insulin sensitizer metformin on many classic and non-classic cardiovascular risk factors (ClinicalTrials.gov NLM Identifier NCT00428311). The overall description and results of the trial have been reported already (Luque-Ramírez *et al.*, 2007b, c).

After giving informed consent, patients were randomized to receive an antiandrogenic oral contraceptive containing 35 µg of ethinyl-estradiol plus 2 mg of cyproterone acetate (Diane³⁵ Diario, Schering España S.A., Madrid, Spain) or 850 mg of metformin (Dianben, Merck Farma y Química S.A., Mollet del Vallés, Spain) daily for 24 weeks. Simple randomization was conducted using blocks of 10 sealed opaque envelopes assigning 5 patients to receive Diane³⁵ Diario and 5 patients to receive metformin. Patients were instructed to maintain a diet containing 25–30 Kcal per kg of body weight daily and moderate physical activity throughout the trial. Patients were submitted to a complete evaluation at baseline and after 12 and 24 weeks of treatment for this and other studies that included anthropometric and laboratory measurements, a 75 g oral glucose tolerance test with measurement of serum insulin and plasma glucose every 30 min for 2 h, and a large number of classic and non-classic cardiovascular risk factors and tests of cardiovascular performance.

Written informed consent was obtained from all the participants in the case–control study and in the clinical trial, and both studies were approved by the local Ethics Committee and by the Spanish Agency of Medicines.

Assays

For the present study, uric acid levels were measured by uricase method using an Abbott Aeroset autoanalyzer (Abbott Laboratories, Abbott Park, IL, USA) with a 0.01 mmol/l limit of detection and mean coefficients of variations <2%. The technical characteristics of the assays employed for plasma glucose and serum hormone measurements have been described elsewhere (Escobar-Morreale *et al.*, 1997, 2000a, b). The composite insulin sensitivity index (Matsuda and DeFronzo, 1999) and the areas under the curve (AUC) for glucose and insulin (Tai, 1994) were calculated from the measurements obtained during the oral glucose tolerance test. The glomerular filtration rate was calculated by the MDRD GFR Calculator (<http://www.mdrd.com>) from the age and creatinine levels of the patients.

Statistical analysis

Data are shown as mean \pm SD or as raw numbers and percentages, as appropriate. For continuous variables, normality was assessed using

the Kolmogorov–Smirnov test and logarithmic or square root transformations were applied as needed to ensure a normal distribution.

The comparison between PCOS patients and controls was analyzed by unpaired *t*-test. We used a general lineal model to evaluate the differences in uric acid levels depending of the grade of obesity and patient or control status, and the possible interaction between these independent variables. The differences between the grades of obesity were then identified by the Bonferroni's test for multiple comparisons.

For discontinuous variables, the χ^2 test or Fisher's exact test were applied as appropriate. The relationship between uric acid concentrations and continuous variables were assessed by Pearson's correlation analysis. Multiple linear regression analysis using a stepwise method (Probability for entry ≤ 0.05 , Probability for removal ≥ 0.10) for the introduction of independent variables was used to identify the main determinants of uric acid levels among the variables showing a statistically significant correlation with this marker.

Treatment effects on serum uric acid concentration were analyzed by repeated-measures general lineal model including the arm of treatment as the between-subjects effect, the visit (baseline, 12 and 24 weeks) as the within-subjects effect, and the interaction between both effects to estimate the differences in the response to each treatment over time. Because 7 patients discontinued metformin for different reasons (Luque-Ramírez *et al.*, 2007b), the results obtained when considering only the patients completing the three visits of the protocol were also confirmed by intention-to-treat analysis assuming no changes in dependent variables at the missing visits with respect to the previous evaluation for patients discontinuing metformin. $P < 0.05$ was considered statistically significant. Analyses were performed using the Statistical Package for the Social Sciences 10 for Macintosh (SPSS Inc, Chicago, IL, USA).

Results

Comparison of serum uric acid levels among PCOS patients and non-hyperandrogenic controls

PCOS patients presented with increased serum androgen levels and a higher degree of insulin resistance—as reflected by increased insulin AUC during the oral glucose tolerance test and a reduced insulin sensitivity index—compared with the control group, yet there were no differences in serum uric acid concentrations between these groups (Table I, Fig. 1). In contrast, weight excess increased serum uric acid levels irrespective of the PCOS or control status of the women studied here, because obese women had significantly higher levels when compared with both overweight and lean subjects (Fig. 1).

Main determinants of serum uric acid levels in premenopausal women

When considering PCOS patients and controls as a whole, serum uric acid levels correlated directly with BMI, waist-to-hip ratio, free androgen index and the insulin AUC during the oral glucose tolerance test, and inversely with the insulin sensitivity index (Table II). On the contrary, when these variables were used to predict serum uric acid levels using stepwise linear regression analysis, only the BMI, which was responsible of 35.4% of the variability observed in serum uric acid concentrations, was retained by the regression model (Table II; Fig. 2A).

Table I. Clinical and biochemical characteristics of the PCOS patients and non-hyperandrogenic control women recruited for the case-control study.

	Patients (n = 40)	Controls (n = 40)	P-value
Age (year)	24.5 ± 5.8	25.6 ± 6.0	0.430
Smokers, n (%)	17 (43)	17 (43)	1.000
BMI (kg/m ²)	29.4 ± 6.3	29.4 ± 6.9	0.966
Waist-to-hip ratio	0.81 ± 0.10	0.78 ± 0.7	0.173
Free androgen index	7.6 ± 5.8	3.9 ± 2.9	<0.001
Androstenedione (nmol/l)	12.9 ± 3.5	8.4 ± 3.1	<0.001
Dehydroepiandrosterone-sulfate (μmol/l)	6.6 ± 2.7	4.4 ± 2.2	<0.001
AUC glucose (mmol/l*120 min)	847 ± 180	826 ± 121	0.542
AUC insulin (pmol/l*120 min)	67 554 ± 38 982	43 864 ± 24 280	0.002
Insulin sensitivity index	4.7 ± 3.7	7.0 ± 4.3	0.002
Uric acid (μmol/l)	256 ± 65	244 ± 65	0.350

Data are expressed as mean ± SD, or raw numbers (percentage). Data were submitted to unpaired *t*-test, Fisher's exact test or χ^2 test, as appropriate. To convert to conventional units, multiply androstenedione by 0.286 (result in ng/ml), dehydroepiandrosterone sulfate by 368 (result in ng/ml), glucose by 18 (result in mg/dl), insulin by 0.144 (result in μU/ml) and uric acid by 0.017 (result in mg/dl). AUC, area under the curve during the oral glucose tolerance test; BMI, body mass index.

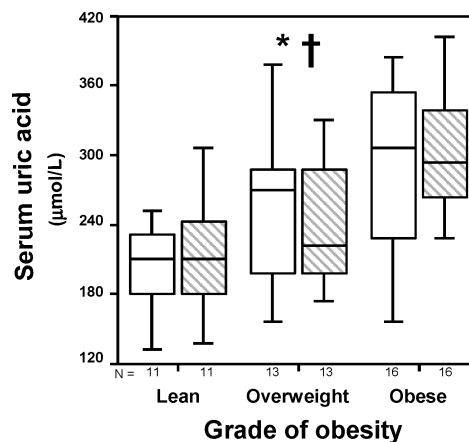


Figure 1: Serum uric acid concentrations in all of the PCOS patients and non-hyperandrogenic controls as a function of obesity.

The box-plot includes the median (horizontal line) and the inter-quartile range (box), and the whiskers indicate the minimum and maximum data values, unless outliers are present in which case the whiskers extend to a maximum of 1.5 times the inter-quartile range. The open boxes correspond to non-hyperandrogenic controls and the patterned boxes correspond to PCOS patients. The figures below the x-axis indicate the number of subjects in each subgroup. * $P < 0.05$ when comparing obese with overweight women, irrespective of having PCOS or being a control. † $P < 0.05$ when comparing obese with lean women, irrespective of having PCOS or being a control. To convert to conventional units, multiply uric acid by 0.017 (result in mg/dl). PCOS, polycystic ovary syndrome.

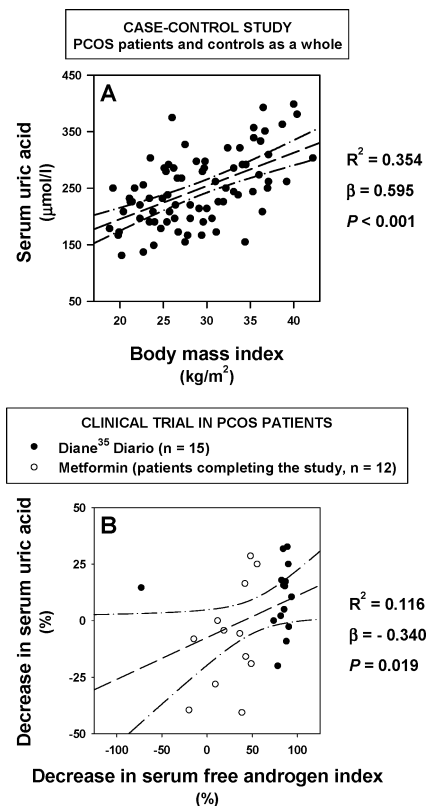
Effects of treatment with either Diane³⁵ Diario or metformin on serum uric acid levels

No differences were observed in the baseline characteristics, including serum uric acid, of the PCOS patients allocated to either treatment with Diane³⁵ Diario or to metformin (Table III). As previously reported, Diane³⁵ Diario was superior to metformin for the control of hyperandrogenism and menstrual disturbances, and increased plasma Apo A-I

Table II. Determinants of serum uric acid concentrations.

Correlation analysis			Stepwise multiple regression analysis		
Variables	<i>r</i>	<i>P</i> -value	Variable retained	Model statistics	<i>P</i> -value
Determinants of serum uric acid levels in the case-control study considering PCOS patients (<i>n</i> = 40) and controls (<i>n</i> = 40) as a whole					
Age	-0.147	0.193	BMI	$R^2 = 0.354$	<0.001
BMI	0.595	<0.001		$\beta = 0.595$	
Waist-to-hip ratio	0.367	0.001			
Free androgen index	0.387	<0.001			
AUC glucose	0.225	0.044			
AUC insulin	0.427	<0.001			
Insulin sensitivity index	-0.466	<0.001			
Determinants of the change (expressed as percentage of baseline values) in serum uric acid levels after treatment with either Diane ³⁵ Diario or metformin in PCOS patients (<i>n</i> = 27)					
Δ BMI (%)	0.187	0.209	Δ Free androgen index (%)	$R^2 = 0.116$	0.019
Δ Waist-to-hip ratio (%)	0.015	0.921		$\beta = 0.340$	
Δ Free androgen index (%)	0.340	0.019			
Δ AUC glucose (%)	0.029	0.845			
Δ AUC insulin (%)	0.189	0.204			
Δ Insulin sensitivity index (%)	0.047	0.755			

Δ, change with respect to baseline, expressed as percentage of initial values; β, standardized regression coefficient; *r*, coefficient of correlation; R^2 , coefficient of determination; BMI, body mass index.

**Figure 2:** Main determinants of serum uric acid levels.

(A) Multiple linear regression analysis using a stepwise method (probability for entry ≤ 0.05 , probability for removal ≥ 0.10) for the introduction of independent variables was used to identify the main determinants of uric acid levels among the variables showing a statistically significant correlation with this marker at baseline. To convert to conventional units, multiply uric acid by 0.017 (result in mg/dl). (B) Multiple linear regression analysis using a stepwise method (probability for entry ≤ 0.05 , probability for removal ≥ 0.10) for the introduction of independent variables was used to identify the main determinants of the decrease in serum uric acid levels (expressed as percentage of initial values) among the changes observed during the study in variables showing a statistically significant correlation with this marker in the whole population of PCOS patients and controls.

and HDL-phospholipids levels, whereas the insulin sensitivity index increased with metformin but did not change with Diane³⁵ Diario (Luque-Ramírez *et al.*, 2007b).

Serum uric acid levels decreased during the 24 weeks of the study in the whole group of PCOS patients (Fig. 3A: Wilks' $\lambda = 0.784$, $F = 3.307$, $P = 0.054$; intention-to-treat analysis: Wilks' $\lambda = 0.787$, $F = 4.186$, $P = 0.025$), yet this decrease was actually caused by the reduction observed in the women treated with Diane³⁵ Diario as demonstrated by the statistically significant interaction of the effect of the visit of evaluation with the arm of treatment (Fig. 3A: Wilks' $\lambda = 0.714$, $F = 4.798$, $P = 0.018$; intention-to-treat analysis: Wilks' $\lambda = 0.681$, $F = 7.250$, $P = 0.003$). Of note, the decrease in serum uric acid levels paralleled that of the free androgen index, which showed a similar interaction between the effect of the visit of evaluation and the arm of treatment (Fig. 3B: Wilks' $\lambda = 0.770$, $F = 3.591$, $P = 0.043$; intention-to-treat analysis: Wilks' $\lambda = 0.684$, $F = 7.160$, $P = 0.003$).

Furthermore, the decrease in serum uric acid concentrations during treatment with Diane³⁵ Diario occurred both in non-obese (BMI < 30.0 kg/m) and in obese (BMI ≥ 30.0 kg/m²) women because a general linear regression model that also considered the absence or presence of obesity as independent variable did not show any statistically significant interaction between the visit of evaluation, the arm of treatment and obesity (Wilks' $\lambda = 0.962$, $F = 0.431$, $P = 0.655$; intention-to-treat analysis: Wilks' $\lambda = 0.952$, $F = 0.733$, $P = 0.489$).

The reduction in serum uric acid levels was also independent from changes in renal function, because the glomerular filtration rate did not change either when considering all patients as a whole (Fig. 3C: Wilks' $\lambda = 0.956$, $F = 0.558$, $P = 0.580$; intention-to-treat analysis: Wilks' $\lambda = 0.959$, $F = 0.671$, $P = 0.518$) or when also considering the interaction of the visit of evaluation with the arm of treatment (Fig. 3C: Wilks' $\lambda = 0.950$, $F = 0.634$, $P = 0.539$; intention-to-treat analysis: Wilks' $\lambda = 0.915$, $F = 1.434$, $P = 0.254$).

Table III. Baseline characteristics of the patients randomized to receive Diane³⁵ Diario or metformin.

	Diane ³⁵ Diario (n = 15)	Metformin (n = 19)	P-value
Age (year)	23.4 ± 5.6	25.1 ± 6.6	0.445
Smokers, n (%)	6 (40)	8 (42)	0.901
BMI (kg/m ²)	29.2 ± 5.7	30.5 ± 6.9	0.563
Waist to hip ratio	0.79 ± 0.06	0.82 ± 0.11	0.292
Creatinine (μmol/l)	70 ± 8	73 ± 7	0.159
Glomerular filtration rate (ml/s*1.73 m ²)	1.6 ± 0.3	1.5 ± 0.2	0.112
Free androgen index	6.8 ± 5.1	7.7 ± 5.4	0.644
Androstenedione (nmol/l)	12.2 ± 2.8	13.6 ± 3.8	0.226
Dehydroepiandrosterone-sulfate (μmol/l)	7.4 ± 2.8	6.1 ± 2.5	0.156
AUC glucose (mmol/l*120 min)	841 ± 126	883 ± 227	0.529
AUC insulin (pmol/l*120 min)	68 915 ± 40 232	70 547 ± 39 475	0.906
Insulin sensitivity index	4.4 ± 3.5	3.8 ± 2.4	0.564
Uric acid (μmol/l)	256 ± 59	268 ± 65	0.558

Data are expressed as mean ± SD, or as raw numbers (percentage). Data were submitted to unpaired *t* test, Fisher's exact test or χ^2 test, as appropriate. To convert to conventional units, multiply creatinine by 0.011 (result in mg/dl), glomerular filtration rate by 60 (result in ml/min/1.73 m²), androstenedione by 0.286 (result in ng/ml), dehydroepiandrosterone sulfate by 368 (result in ng/ml), glucose by 18 (result in mg/dl), insulin by 0.144 (result in μ U/ml) and uric acid by 0.017 (result in mg/dl). BMI, body mass index.

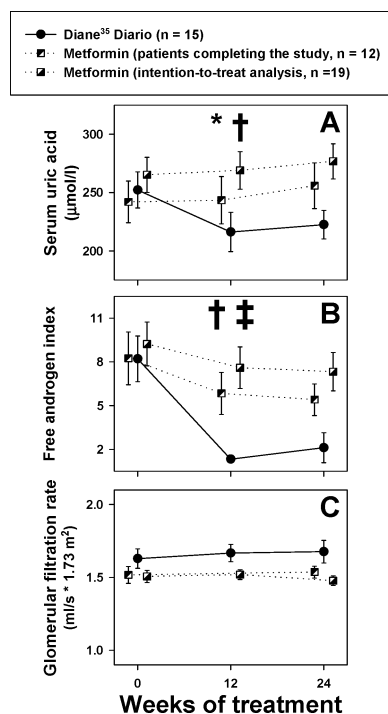


Figure 3: Change in serum uric acid concentration (A), free androgen index (B) and glomerular filtration rate (C) during treatment of PCOS with Diane³⁵ Diario or metformin. Data are mean ± SEM.

* $P < 0.05$ for the decrease with respect of baseline values in the whole group of patients, irrespective of the arm of treatment, only in the intention-to-treat analysis. † $P < 0.05$ for the interaction between the arms of treatment, in both analysis of patients who completed the study and in the intention-to-treat analysis. ‡ $P < 0.05$ for the decrease with respect of baseline values in the whole group of patients, irrespective of the arm of treatment, in both analysis of patients who completed the study and in the intention-to-treat analysis. To convert to conventional units, multiply uric acid by 0.017 (result in mg/dl).

At the end of the study we assessed again the relationships between the serum acid concentrations and the percentage change observed with respect to baseline values in the BMI, waist-to-hip ratio, free androgen index, insulin AUC during the oral glucose tolerance test and in the insulin sensitivity

index. Correlation and regression analysis showed that the decrease in the free androgen index was the main determinant of serum uric acid levels after treatment with either metformin or Diane³⁵ Diario (Table II; Fig. 2B).

Discussion

Our present results confirm that serum uric acid levels are not actually increased in PCOS patients when compared with non-hyperandrogenic women when adequately matched for age, BMI and grade of obesity. On the contrary, obesity was clearly associated with an increase in serum uric acid levels both in PCOS patients and in non-hyperandrogenic controls, and this occurred despite PCOS patients being clearly insulin resistant when compared with the controls. Together with BMI explaining in as much as 35.4% of the variability in the serum uric acid concentrations of premenopausal women, our present results suggest that obesity, and not insulin resistance (Clausen *et al.*, 1998), is the major determinant of serum uric acid levels in premenopausal women. We might speculate that the different prevalences of obesity among the PCOS and control populations influenced the controversial results reported by earlier studies (Quiñonez Zarza *et al.*, 2000; Yarali *et al.*, 2001).

Although most of the women studied here had normal serum uric acid concentrations, the influence of obesity on these levels is important because increasing serum uric acid levels associates increased cardiovascular mortality even with values within the normal range (Fang and Alderman, 2000; Niskanen *et al.*, 2004). Our present results further exemplify the contribution of obesity to the cardiovascular risk associated with PCOS, because obesity appears to be the main factor responsible for the association of PCOS with classic cardiovascular risk factors, such as hypertension (Luque-Ramírez *et al.*, 2007a), and with non-classic markers such as serum uric acid levels and serum markers of low-grade chronic inflammation (Escobar-Morreale *et al.*, 2003).

Even when the stepwise multiple regression model retained only the BMI as the main factor responsible for the increased

serum uric acid levels in our premenopausal women, these levels also correlated with serum androgens and with indexes of abdominal adiposity and insulin resistance, suggesting a perhaps less important participation of these factors in the regulation of serum uric acid concentrations.

The well-known association of increased serum uric acid levels, obesity and insulin resistance is partly explained by the inhibitory action of hyperinsulinism on the renal excretion of uric acid (Quiñones Galvan *et al.*, 1995). Yet androgens might influence also serum uric acid levels to some extent, although as a group our PCOS patients did not show increased uric acid levels despite their obvious hyperandrogenism. The possible relationship between androgens and serum uric acid concentrations is supported by the finding of higher serum uric acid concentrations in men compared with women (Fang and Alderman, 2000), by the correlation between serum androgen and uric acid concentrations in the female population (Mantzoros *et al.*, 1995), and by animal experiments showing that androgens may increase serum uric acid levels by inducing the hepatic metabolism of purines (Vizzotto *et al.*, 1996; Marinello *et al.*, 2004).

In conceptual agreement with a role of androgens in the regulation of serum uric acid levels in premenopausal women, only the antiandrogenic oral contraceptive pill Diane³⁵ Diario, and not the insulin sensitizer metformin, decreased uric acid concentrations significantly in our PCOS patients in parallel to the decrease in serum androgens, exemplified here by the decrease in the free androgen index. Furthermore, at the end of the clinical trial the serum uric acid concentrations attained correlated mainly with the degree of amelioration of hyperandrogenemia, which was much more marked with the contraceptive than with metformin (Luque-Ramírez *et al.*, 2007b). Moreover, the decrease in serum uric acid levels during treatment with Diane³⁵ Diario was observed both in obese and in non-obese women, suggesting that the relationship between androgens and uric acid is mediated by effects that are independent from obesity. In this regard, synthetic estrogens have uricosuric effects that might contribute to our present finding (Adamopoulos *et al.*, 1977). And finally, the lack of effect of metformin on uric acid, despite the improvement observed in insulin sensitivity with its use in PCOS patients (Luque-Ramírez *et al.*, 2007b), further indicates that insulin resistance is not the major regulator of serum uric acid concentrations in these women.

It could be argued that the magnitude of the changes in serum uric acid levels during treatment with Diane³⁵ Diario in our PCOS patients was small and occurred within the normal range in most cases. Yet increases in uric acid concentrations as small as 59 $\mu\text{mol/l}$ increase the frequency of cardiovascular events and ischemic cardiopathy in epidemiological studies (Fang and Alderman, 2000). Moreover, 29% of the decrease in the primary outcome—cardiovascular death, fatal or nonfatal myocardial infarction, fatal or nonfatal stroke—observed in the arm of treatment with losartan in the LIFE study was attributed to the reduction observed in serum uric acid levels secondary to the uricosuric properties of this drug (Hoiegggen *et al.*, 2004), highlighting the possible cardiovascular benefits of controlling and reducing uric acid concentrations. Nevertheless, although classic and non-classic

cardiovascular risk factors cluster in PCOS patients, it must be highlighted that there is no convincing evidence at present of increased cardiovascular events in these women (Wild *et al.*, 2000; Wild, 2002; Legro, 2003).

Our study, however, was not free of limitations. First, because we did not study ovarian morphology in our PCOS patients, our present results should only be applied to the more severe hyperandrogenic PCOS phenotypes, and cannot be extrapolated to the milder PCOS phenotype consisting of oligo-ovulation and polycystic ovarian morphology that does not associate with substantial clinical or biochemical hyperandrogenism (The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group, 2004). Second, our study does not permit any conclusion about the possible changes in serum uric acid levels after life-style modification and weight loss because patients were instructed to maintain a maintenance diet containing 25–30 Kcal per kg of body weight daily and moderate physical activity throughout the trial, explaining why weight and BMI did not change significantly during the trial (Luque-Ramírez *et al.*, 2007b). Considering the major influence that the BMI exerted on serum uric acid concentrations in the present case–control study, it is plausible that weight loss reduces serum uric acid levels also in PCOS patients, as has been shown in the general population (Nicholls and Scott, 1972; Scott and Sturge, 1977).

The fact that metformin did not change serum uric acid levels in our patients does not invalidate the use of this drug in PCOS patients, especially when previous reports of this clinical trial demonstrated amelioration of insulin resistance (Luque-Ramírez *et al.*, 2007b), and a decrease in serum ferritin levels (Luque-Ramírez *et al.*, 2007c) and daytime blood pressure (submitted for publication) with metformin, benefits that were not observed with Diane³⁵ Diario. Actually, these differential effects of oral contraceptives and insulin sensitizers on the different classic and non-classic cardiovascular risk factors associated with PCOS and obesity highlight the importance of tailoring pharmacological treatment to the clinical characteristics and risk factors present in each individual PCOS patient.

In summary, our present results demonstrate that, as occurs with the abnormalities in blood pressure regulation (Luque-Ramírez *et al.*, 2007a), obesity is the major factor responsible for serum uric acid levels in PCOS patients. Yet androgen excess also plays a significant role, considering that antagonizing its effects by the administration of an antiandrogenic oral contraceptive results in a significant decrease in serum uric acid concentrations, an effect that is not observed with the use of the insulin sensitizer metformin. This newly recognized beneficial effect should be added to the previously known efficacy of oral contraceptives for the control of PCOS symptoms, and the use of these drugs should be considered even in obese patients who are those at higher risk for increased serum uric acid levels.

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