

## The Efficacy of 24-Month Metformin for Improving Menses, Hormones, and Metabolic Profiles in Polycystic Ovary Syndrome

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**Context:** The long-term effects of metformin in women with polycystic ovarian syndrome (PCOS) are inadequately studied.

**Objective:** The effects of metformin on women with PCOS during 24 months with respect to menses, hormones, and metabolic profiles are assessed.

**Design:** Prospective cohort.

**Setting:** A reproductive endocrinology clinic in a university-affiliated medical center.

**Patients:** One hundred nineteen women with PCOS, defined by the Rotterdam criteria, were enrolled.

**Intervention:** Metformin was given daily for 24 months.

**Main Outcome Measures:** The primary outcome was the proportion of patients with regular menstruation during treatment. Changes in anthropometric, hormonal, and metabolic parameters were also assessed. Analyses were performed using segmented regression analysis with a generalized estimating equation methodology. Outcomes are expressed as magnitude of change from the baseline.

**Results:** Both overweight (OW) and normal-weight (NW) women with PCOS had increased menstrual frequency and decreased body mass index (BMI), testosterone, and luteinizing hormone levels in the first 6 months. Further stratification showed that NW women exhibiting elevated testosterone at baseline had the largest magnitude of improvement at 6 months [odds ratio (OR), 7.21; 95% confidence interval (CI), 2.35 to 22.17], whereas OW patients with normal testosterone were most likely to achieve normal menses at 12 months (OR, 0.63; 95% CI, 0.47 to 0.77).

**Conclusions:** Metformin was associated with improvements in the menstrual cycle and most hormonal profiles in OW and NW women with PCOS during 24 months of treatment. Most parameters reached maximal response and steady-state after 6 months. Phenotypic differences in baseline BMI and testosterone level can be used as patient selection criteria or treatment prognostics. (*J Clin Endocrinol Metab* 103: 890–899, 2018)

**W**omen with polycystic ovarian syndrome (PCOS) are characterized by hyperandrogenism, anovulation, and polycystic ovarian morphology on ultrasound (1). There is ample evidence to indicate that PCOS is not restricted to the ovaries, but manifests as system-wide aberrations, such as dyslipidemia, insulin resistance, abdominal obesity, and hypertension (2). The relationship between PCOS and insulin resistance is well documented (3). Although the primary etiology remains unclear, multiple factors contribute to the occurrence of insulin resistance in PCOS, including obesity (4), hyperandrogenism (3), and a defect in the cellular insulin signaling pathway (5). Hyperinsulinemia in response to the insulin resistance further exacerbates the hormonal and ovulatory dysfunctions associated with PCOS (6).

Metformin, an insulin sensitizer, has been evaluated as a therapeutic option for reducing insulin resistance in women with PCOS (7). Initial results from noncontrolled studies demonstrate that metformin has beneficial effects on hyperinsulinemia, hyperandrogenemia, ovarian steroidogenesis, menstrual cycles, and pregnancy rates (7–10). More recently, several prospective randomized studies and a meta-analysis have confirmed the positive effects of metformin on metabolic derangements, hyperinsulinemia, hyperandrogenemia, blood pressure, and clinical pregnancy rates (11–19).

Concerning the effects of metformin on restoring normal menses, data from noncontrolled studies are promising (7), but the benefits have not been consistently reproduced in controlled studies (13–19). This variation in results may be due to the substantial heterogeneity in treatment duration across the different studies, which ranged from 3 months to a year (13, 16, 19). Furthermore, past studies were limited to obese and/or hyperandrogenic patients (13, 15, 16), rather than conforming to the more comprehensive Rotterdam criteria (1). Despite the fact that insulin resistance tends to manifest in obese women with PCOS (20), there is evidence to indicate that metformin is effective in nonselected women with PCOS, as controlled studies that did not select for obesity displayed good efficacy in the restoration of normal menses (17, 19).

Therefore, evaluating the effect of metformin on the menstrual cycle in nonselected women with PCOS in a longitudinal design is needed, preferentially with a focus on phenotypic differences. In particular, attention should be paid to the nonobese, normal androgenic phenotype for being underrepresented in past studies and being the more prevalent phenotype in Asians (21). In this study, we compared the treatment response, including changes in the menstrual cycle, hormonal, metabolic, and anthropometric profiles, in overweight (OW) and normal-

weight (NW) women with PCOS treated with metformin during the course of 24 months. We also investigated the efficacy of menstrual cycle restoration with respect to baseline body mass index (BMI) and androgen phenotypes. The repeatedly measured, longitudinal data were analyzed using the generalized estimating equation (GEE) methodology in a segmented regression model (22), which assesses for changes in clinical parameters in response to treatment duration.

## Material and Methods

### Subjects

One hundred nineteen women with PCOS were enrolled into the study at the National Taiwan University Hospital from 2008 to 2013. All patients initially sought evaluation at our reproductive endocrinology clinic for problems relating to menstrual irregularities and/or symptoms of hyperandrogenism. Diagnosis of PCOS was based on the criteria proposed at the Rotterdam revised consensus meeting (1), which requires at least two of the following phenotypes: the presence of oligomenorrhea or amenorrhea; clinical/biochemical hyperandrogenism; and polycystic ovaries as visualized by pelvic ultrasonography (23). Oligomenorrhea was defined as less than eight spontaneous menstrual cycles per year, which averaged to a cycle interval greater than 45 days in the 3 years preceding enrollment, in alignment with our previous study (24). Hyperandrogenism was defined as a serum testosterone level  $\geq 0.7$  ng/mL, in accordance with the published upper limit for normal females using the direct radioimmunoassay methodology (25), and/or clinical hyperandrogenism, manifesting as persistent acne, hirsutism (a Ferriman–Gallwey score  $> 8$ ), or androgenic alopecia. Women with hyperprolactinemia, thyroid dysfunction, Cushing syndrome, congenital adrenal hyperplasia, adrenal tumors, or other virilizing ovarian tumors were excluded. Additional exclusion criteria included ongoing pregnancy, pregnancy in the past year, documented use of oral contraceptives or other medications that affect the hypothalamic–pituitary–ovarian axis in the previous 6 months, any concomitant major systemic disease, such as an autoimmune disease, malignancy, central nervous system disease, or prior chemotherapy or immunosuppressive agent use. The Institutional Review Board of National Taiwan University Hospital approved this study, and signed informed consents were obtained from all patients or their legal guardians before commencing data collection.

### Protocol and data collection

Clinical evidence of hyperandrogenism, oligomenorrhea/amenorrhea, polycystic morphology, and baseline anthropometric measurements were recorded at patient enrollment. During the 24-month study period, patients were prescribed 500 mg of metformin/d (Loditon; Standard Chem and Pharm, Tainan, Taiwan) in the first month, followed by 1000 mg/d in the second month and 1500 mg/d from the third to 24th month. A decrease to 1000 mg/d dose was allowed in patients reporting intolerable gastrointestinal side effects. Patients were evaluated for blood pressure, anthropometric measurements, hormonal profiles, and metabolic profiles at enrollment (M0) and after month 3 (M3), month 6 (M6), month 12 (M12), and month 24 (M24) of metformin use.

At baseline, overnight fasting blood samples were collected before hormone-induced withdrawal bleeding in amenorrheic women, and in the early follicular phase in women with spontaneous menses. The collection of blood samples were repeated at each of the four aforementioned time points. The procedure used for blood sample collection has been previously described in detail (26). BMI was calculated as weight (kg) divided by squared height ( $m^2$ ), and the OW subgroup was defined as a BMI  $\geq 25$  kg/ $m^2$ , which corresponds to an OW status in the general population (27) and obesity in the Asian population (28).

Menstrual regularity at the time of enrollment was assessed using self-reported menstrual intervals of the past 3 years, which were based on diary review. Normal menstrual cycle was defined as a cycle interval  $\geq 21$  days and  $\leq 45$  days. This more stringent definition of the normal menstrual cycle was used due to the inclusion of adolescents in this study (29). During the study period, a normal menstrual cycle was defined as  $\geq 2$  ovulatory menses in a 3-month interval, consistent with the aforementioned menstrual interval of 45 days. Ovulatory menses was defined as vaginal bleeding following either a biphasic basal body temperature chart or elevated serum progesterone, which was checked weekly in patients with non-phasic basal body temperatures.

## Assay methods

Plasma glucose concentrations were measured using an autoanalyzer (Toshiba TBA-120 FR; Toshiba, Tokyo, Japan). The serum levels of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides were measured using a biochemical autoanalyzer (Toshiba TBA-200FR). Serum insulin levels were determined using a microparticle enzyme immunoassay in an AxSYM system (Abbott Laboratories, Dainabot Co., Tokyo, Japan). Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and progesterone levels were measured using indirect chemiluminescence (VitrosEci; Ortho Clinical Diagnostics, Rochester, NY). Serum sex hormone-binding globulin (SHBG) levels were measured using electrochemiluminescence (Elecys 2010; Roche Diagnostics, Mannheim, Germany). Serum testosterone and dehydroepiandrosterone sulfate (DHEA-S) levels were measured using radioimmunoassays (Diagnostic Systems Laboratories, Webster, TX). The free androgen index (FAI) was calculated using the equation  $FAI (\%) = \text{testosterone (ng/mL)} \times 3.47 \times 100 / SHBG \text{ (nmol/L)}$ , and the homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as  $HOMA-IR = [\text{glucose (mg/dL)} \times 0.05551] \times \text{insulin (IU/mL)} / 22.5$  (30). The LH/FSH ratio was calculated as LH (mIU/mL) divided by FSH (mIU/mL). A normal testosterone (NT) level was defined as a serum level  $< 0.7$  ng/mL (25), whereas an elevated testosterone (ET) level was defined as a serum level  $\geq 0.7$  ng/mL. The intra-assay and interassay coefficients of variation of the aforementioned assays were all  $< 10\%$ .

## Statistical analysis

For comparisons between the two BMI subgroups at baseline, the two-sample Wilcoxon rank-sum test and  $\chi^2$  test were applied. The numerical variables are presented as the median, the first and the third quartiles, and binary variables, such as the proportion of patients with normal menstrual cycle or NT, are

presented as percentages. All statistical tests used a two-sided significance level of 0.05.

For regression analysis of the repeatedly measured variables with respect to duration of metformin use, the GEE approach was performed with stratification by BMI and/or testosterone phenotype. Normal menstrual cycle, recorded as a binary outcome variable, was expressed as the log odds of having the outcome. Quantitative outcome variables, such as FAI, SHBG, LH, FSH, the LH/FSH ratio, DHEA-S, insulin, and HOMA-IR, were expressed at each time point as the log ratios to the baseline values. Treatment periods were designated as M0/M3, M3/M6, M6/M12, and M12/M24, and a segmented regression analysis during the four treatment periods was performed by fitting a separate line segment to each interval and connecting all of these line segments. Line segments were combined when two adjacent time periods significantly exhibited the same time trend effect. Besides evaluating the effects of treatment duration and OW individually, interactions between treatment interval and OW were considered as explanatory variables in the regression models. The association between an outcome and an explanatory variable was assessed as the odds ratio (OR) or the ratio with a 95% confidence interval (CI).

The link function, which describes the relationship between the mean responses of the distribution function and the linear explanatory variables, was the logit link function for the binary variable and the identity link function for quantitative variables. In this study, we adopted an unstructured working correlation matrix to describe the associations between the five repeated measurements of the same outcome variable. The selection of an optimal GEE model for each outcome variable was based on the preference for smaller values of the quasi-likelihood under the independence model criterion (31), which is analogous to the Akaike information criterion (32). The estimates of regression coefficients in a selected model were considered jointly significant and not necessarily individually significant.

Predicted values and their corresponding 95% CIs at each time point were calculated by performing an inverse transformation of the associated link function on the log odds or log ratio calculated from the GEE model coefficients. The results for the binary variable normal menstrual cycle were presented as the predicted proportions with normal menses and the ORs of having normal menses in relationship to the baseline. An OR  $> 1$  indicates an improvement in the proportion of patients with regular menstrual cycles. The results for the quantitative variables were reported as the predicted ratios in relationship to the baseline. A ratio of  $< 1$  indicates a decrease in the value. All analyses were performed using R (version 3.3.1; R Foundation for Statistical Computing, Vienna, Austria), and the GEE analyses were performed using the `geeglm` function in the `geepack` package (33).

## Results

### Baseline characteristics

At study entry, the percentages of the 119 women with PCOS that fulfilled the diagnostic criteria for hyperandrogenism/anovulation, hyperandrogenism/polycystic ovarian morphology, anovulation/polycystic ovarian morphology, and all three were 17.6%, 12.6%, 20.2%, and 49.6%, respectively. Forty-seven of the patients were OW, and 72 were NW. The baseline (M0)

anthropometric, hormone, and metabolic characteristics are shown in Table 1. As expected, baseline measurements such as waist circumference, body weight, FAI, glucose, insulin, HOMA-IR, triglycerides, and low-density lipoprotein were significantly higher in the OW subgroup. SHBG, FSH, LH, the LH/FSH ratio, and high-density lipoprotein cholesterol levels were significantly higher in the NW subgroup. The proportion of patients with normal menstrual cycles was higher in the OW subgroup than in the NW subgroup.

### Changes in ovulatory menstrual cycles after 24 months of metformin treatment

Patients were stratified by BMI or testosterone status, and menstrual regularity was assessed by segmented regression analysis. Details of the methodology, formula, and regression coefficients for the GEE segmented logistic regression model are included in Supplemental Appendix A, Supplemental Table 1, and Supplemental Table 2, respectively. Results are presented as the predicted proportions of patients with normal menstrual cycles at each time point (Fig. 1; Table 2) and ORs of having normal menses in relationship to the baseline (Table 3).

Regardless of whether the patients were stratified by BMI or testosterone status, the proportion of patients with normal menstrual cycles after receiving metformin was generally higher than the proportion at the baseline (Fig. 1; Tables 2 and 3). When stratified by BMI, the

improvement in the NW subgroup was significant at M3 (Table 3) and remained significant up to M24 (Table 2). In contrast, when stratified by testosterone levels, both subgroups exhibited significant improvements in the first 6 months. The NT subgroup continued this trend at a constant slope from M3 to M24 [slope estimate = 0.033, standard error (SE) = 0.014,  $P < 0.05$ , Supplemental Table 2], whereas the ET subgroup diverged non-significantly from this trend after M6 (slope estimate = 0.088, SE = 0.073, Supplemental Table 2).

In a further analysis, BMI and testosterone stratifications were combined, resulting in four subgroups: NW-NT, NW-ET, OW-NT, and OW-ET. Analyses demonstrated a lower proportion of women with spontaneous menstruation in the NW-ET subgroup at M0, but a higher rate of improvement from M0 to M6 (Fig. 2; Table 3). The NW-ET subgroup was also associated with the largest overall improvement over the baseline (OR, 7.21; 95% CI, 2.35 to 22.17). All four subgroups exhibited improvements at 6 months, with the percentages of women with normal menses ranging from 42% to 47% (Fig. 2; Table 2). With the exception of the OW-NT subgroup, no further changes in menstrual pattern was seen after 6 months. The OW-NT subgroup continued to exhibit improvements up to M12, and had the highest predicted proportion of patients with normal menstrual cycles at the end of the 24-month period (OR, 0.63; 95% CI, 0.47 to 0.77).

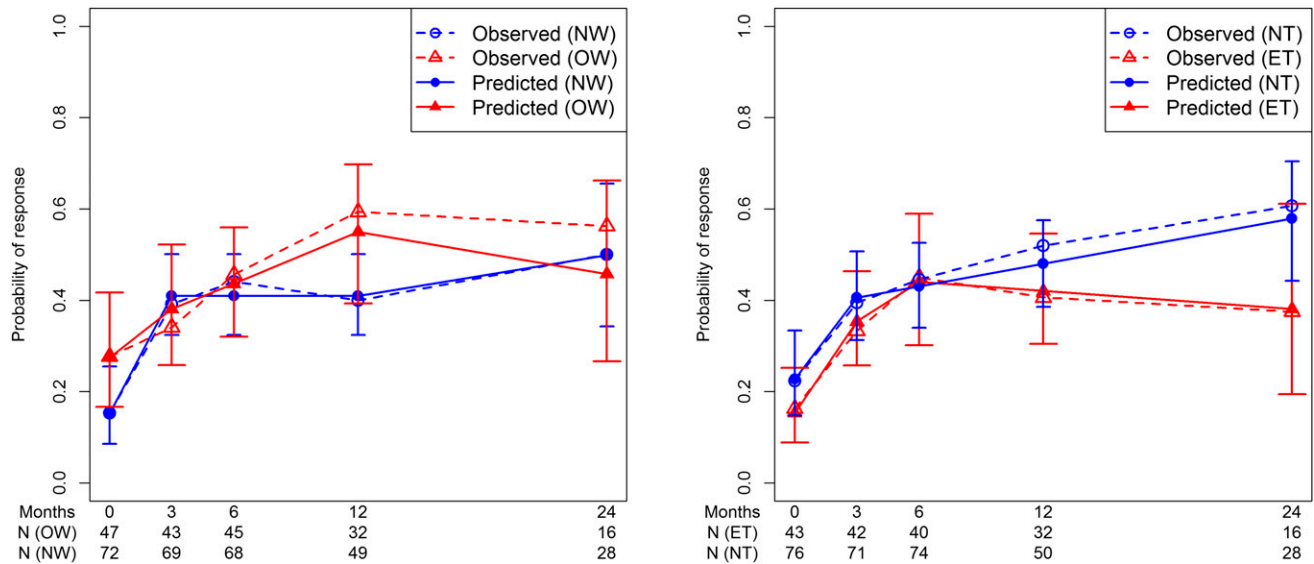
**Table 1. Patient Characteristics at Baseline**

Characteristics	NW (n = 72): Median [Q1, Q3]	OW (n = 47): Median [Q1, Q3]	P Value
Age, y	24.5 [21.0, 27.0]	25.0 [19.0, 29.5]	0.645
Waist, cm	78.8 [74.9, 83.1]	98.0 [91.3, 105.5]	<0.001 <sup>a</sup>
BW, kg	54.3 [50.2, 58.0]	77.6 [68.7, 84.2]	<0.001 <sup>a</sup>
Testosterone, ng/mL	0.6 [0.4, 0.7]	0.6 [0.5, 0.9]	0.139
SHBG, nmol/L	39.0 [30.9, 52.8]	18.3 [14.8, 25.1]	<0.001 <sup>a</sup>
FAI, %	4.7 [3.2, 7.2]	12.2 [7.9, 15.0]	<0.001 <sup>a</sup>
LH, mIU/mL	13.4 [10.6, 16.1]	8.4 [5.2, 10.6]	<0.001 <sup>a</sup>
FSH, mIU/mL	6.6 [5.5, 7.8]	6.0 [5.2, 6.7]	0.027 <sup>a</sup>
LH/FSH ratio	2.0 [1.6, 2.7]	1.3 [1.0, 1.7]	<0.001 <sup>a</sup>
DHEA-S, µg/dL	268.3 [189.0, 354.9]	255.7 [167.6, 324.5]	0.369
Insulin, IU/mL	2.8 [2.0, 4.2]	14.4 [7.7, 22.6]	<0.001 <sup>a</sup>
Glucose, IU/mL	80.5 [77.0, 84.3]	85.0 [80.0, 89.0]	0.001 <sup>a</sup>
HOMA-IR	0.5 [0.4, 0.8]	3.0 [1.5, 5.0]	<0.001 <sup>a</sup>
LDL-C, mg/dL	93.5 [79.8, 114.3]	116.0 [95.0, 135.0]	0.001 <sup>a</sup>
HDL-C, mg/dL	59.0 [52.0, 68.0]	46.0 [41.0, 52.0]	<0.001 <sup>a</sup>
TG, mg/dL	65.5 [49.0, 87.5]	97.0 [75.5, 142.0]	<0.001 <sup>a</sup>
Menstrual cycle interval, d	135 [75, 360]	81 [45, 172.5]	0.031 <sup>a</sup>
Normal menstrual cycle	7/72 (9.7%)	12/47 (25.5%)	0.021 <sup>a</sup>
Normal testosterone	49/72 (68.1%)	27/47 (57.4%)	0.239

Q1 and Q3 denote the first and third quartiles, respectively. Normal menstrual cycle was defined as a menstrual interval  $\leq 45$  days, and normal testosterone level was defined as testosterone level  $< 0.7$  ng/mL. Values are expressed as the median with the first and the third quartiles and proportions (percentage) as appropriate. NW is defined as BMI  $< 25$  kg/m<sup>2</sup>, and OW is defined as BMI  $\geq 25$  kg/m<sup>2</sup>.

Abbreviations: BW, body weight; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides.

<sup>a</sup> $P < 0.05$ , by Wilcoxon rank-sum test.



**Figure 1.** Predicted and observed proportions of patients with normal menstrual cycles at each time point with 95% CIs in (left) BMI and (right) testosterone stratified analyses. NW is defined as BMI <25 kg/m<sup>2</sup>, OW as BMI ≥25 kg/m<sup>2</sup>, NT as serum testosterone <0.7 ng/mL, and ET as serum testosterone ≥0.7 ng/mL. N indicates sample size at each follow-up time.

### Longitudinal changes in other phenotypes after 24 months of metformin treatment in OW and NW women

Changes in hormonal and metabolic variables in response to metformin treatment are presented as ratios to the baseline at M3, M6, M12, and M24 in Fig. 3 and Table 4. The box plots for each variable and the regression coefficients of the GEE model are summarized in Supplemental Appendix B and Supplemental Table 3, respectively. Compared with the baseline, BMI, testosterone, FAI, LH levels, and LH/FSH ratios were all significantly lower at M24 in the metformin-treated patients in both subgroups. A significant decline in BMI, testosterone, FAI, and LH levels was observed in response to metformin at M6, but there were no further significant changes after M6 in most parameters. The only exception was a decline in FAI in the NW subgroup from M12 to M24 (Fig. 3; Supplemental Table 3). There were

significant differences between the NW and OW subgroups during the first 3 months with respect to BMI, testosterone, FAI, and LH levels. At M3, significantly greater changes in BMI, testosterone, and FAI were associated with the OW status, whereas there was a significantly larger decline in LH in the NW subgroup (Fig. 3; Table 4).

Compared with M0, predicted SHBG levels were significantly lower at M12, but higher than the baseline at M24 in the NW subgroup (Fig. 3; Table 4). In contrast, changes in SHBG from M12 to M24 were nonsignificant in the OW subgroup. The predicted DHEA-S levels were not significantly different between the two BMI subgroups and exhibited an increasing trend from M0 to M12 that reversed in the second year (slope estimate = -0.01, SE = 0.003, P < 0.001, Supplemental Table 3), and eventually returned to baseline levels at M24. Both insulin and HOMA-IR significantly decreased in the OW

**Table 2. Proportions of Patients With Regular Menstrual Cycles Are Presented With the Corresponding 95% CI, as Calculated From the GEE Models**

Stratification	Subgroup	M0	M3	M6	M12	M24
BMI	NW	0.15 [0.09, 0.25]	0.41 [0.32, 0.50]	0.41 [0.32, 0.50]	0.41 [0.32, 0.50]	0.50 [0.34, 0.66]
	OW	0.28 [0.17, 0.42]	0.38 [0.26, 0.52]	0.44 [0.32, 0.56]	0.55 [0.39, 0.70]	0.46 [0.27, 0.66]
Testosterone	NT	0.23 [0.15, 0.33]	0.41 [0.31, 0.51]	0.43 [0.34, 0.53]	0.48 [0.39, 0.58]	0.58 [0.44, 0.70]
	ET	0.15 [0.09, 0.25]	0.35 [0.26, 0.46]	0.44 [0.30, 0.59]	0.42 [0.31, 0.55]	0.38 [0.19, 0.61]
BMI and testosterone	NW-NT	0.23 [0.17, 0.31]	0.34 [0.27, 0.42]	0.47 [0.35, 0.59]	0.47 [0.35, 0.59]	0.47 [0.35, 0.59]
	NW-ET	0.10 [0.05, 0.19]	0.22 [0.15, 0.32]	0.43 [0.28, 0.60]	0.43 [0.28, 0.60]	0.43 [0.28, 0.60]
	OW-NT	0.23 [0.17, 0.31]	0.32 [0.24, 0.41]	0.42 [0.32, 0.53]	0.63 [0.47, 0.77]	0.63 [0.47, 0.77]
	OW-ET	0.23 [0.17, 0.31]	0.32 [0.23, 0.42]	0.42 [0.25, 0.61]	0.42 [0.25, 0.61]	0.42 [0.25, 0.61]

NW is defined as BMI <25 kg/m<sup>2</sup>, OW as BMI ≥25 kg/m<sup>2</sup>, NT as serum testosterone <0.7 ng/mL, and ET is defined as serum testosterone ≥0.7 ng/mL.

**Table 3. OR of Having Regular Menstrual Cycles During Treatment in Relationship to Baseline Are Presented With the Corresponding 95% CI, as Calculated From the GEE Models**

Stratification	Subgroup	M0	M3	M6	M12	M24
BMI	NW	1	3.87 <sup>a</sup> [2.10, 7.14]	3.87 <sup>a</sup> [2.10, 7.14]	3.87 <sup>a</sup> [2.10, 7.14]	5.55 <sup>a</sup> [2.55, 12.08]
	OW	1	1.63 [0.77, 3.45]	2.04 <sup>a</sup> [1.05, 3.98]	3.23 <sup>a</sup> [1.56, 6.66]	2.23 [0.76, 6.56]
Testosterone	NT	1	2.32 <sup>a</sup> [1.44, 3.74]	2.57 <sup>a</sup> [1.61, 4.09]	3.13 <sup>a</sup> [1.93, 5.08]	4.67 <sup>a</sup> [2.45, 8.90]
	ET	1	3.03 <sup>a</sup> [1.77, 5.18]	4.35 <sup>a</sup> [1.89, 10.02]	4.02 <sup>a</sup> [1.79, 8.97]	3.39 <sup>a</sup> [1.04, 11.09]
BMI and testosterone	NW-NT	1	1.71 <sup>a</sup> [1.28, 2.27]	2.92 <sup>a</sup> [1.65, 5.17]	2.92 <sup>a</sup> [1.65, 5.17]	2.92 <sup>a</sup> [1.65, 5.17]
	NW-ET	1	2.69 <sup>a</sup> [1.53, 4.71]	7.21 <sup>a</sup> [2.35, 22.17]	7.21 <sup>a</sup> [2.35, 22.17]	7.21 <sup>a</sup> [2.35, 22.17]
	OW-NT	1	1.55 <sup>a</sup> [1.30, 1.84]	2.39 <sup>a</sup> [1.69, 3.37]	5.71 <sup>a</sup> [2.87, 11.34]	5.71 <sup>a</sup> [2.87, 11.34]
	OW-ET	1	1.54 <sup>a</sup> [1.01, 2.36]	2.38 <sup>a</sup> [1.02, 5.56]	2.38 <sup>a</sup> [1.02, 5.56]	2.38 <sup>a</sup> [1.02, 5.56]

NW is defined as BMI <25 kg/m<sup>2</sup>, OW as BMI ≥25 kg/m<sup>2</sup>, NT as serum testosterone <0.7 ng/mL, and ET is defined as serum testosterone ≥0.7 ng/mL. <sup>a</sup>*P* < 0.05.

subgroup during the first year, but these improvements did not persist beyond M12 and returned to the baseline level by M24. The slope increase from M12 to M24 was considered significant (slope estimate = 0.039, SE = 0.016, *P* < 0.05, Supplemental Table 3). No discernible change in insulin and HOMA-IR was seen in the NW subgroup during the whole treatment period.

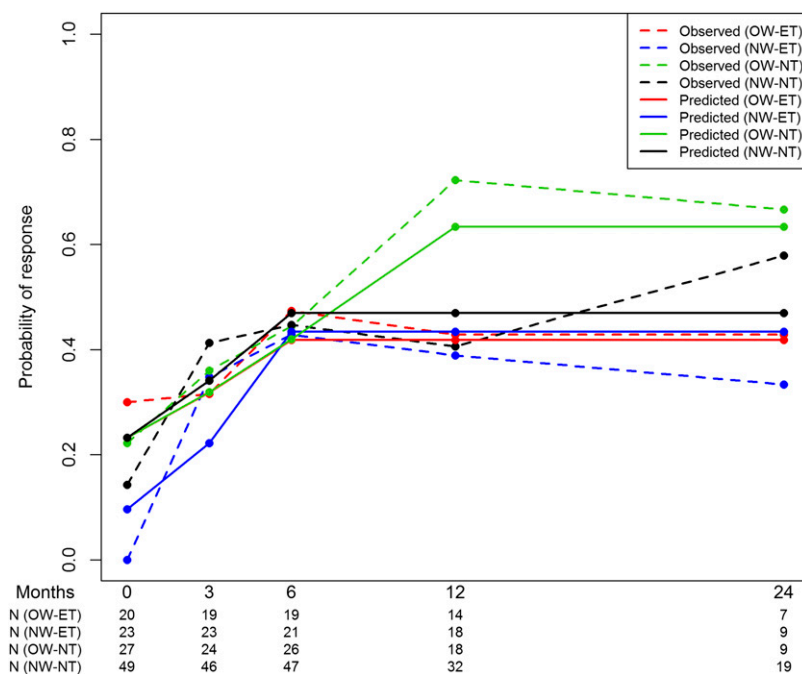
## Discussion

The results of our study demonstrate that there are differences in treatment response to metformin between different BMI/testosterone phenotypes. Regarding

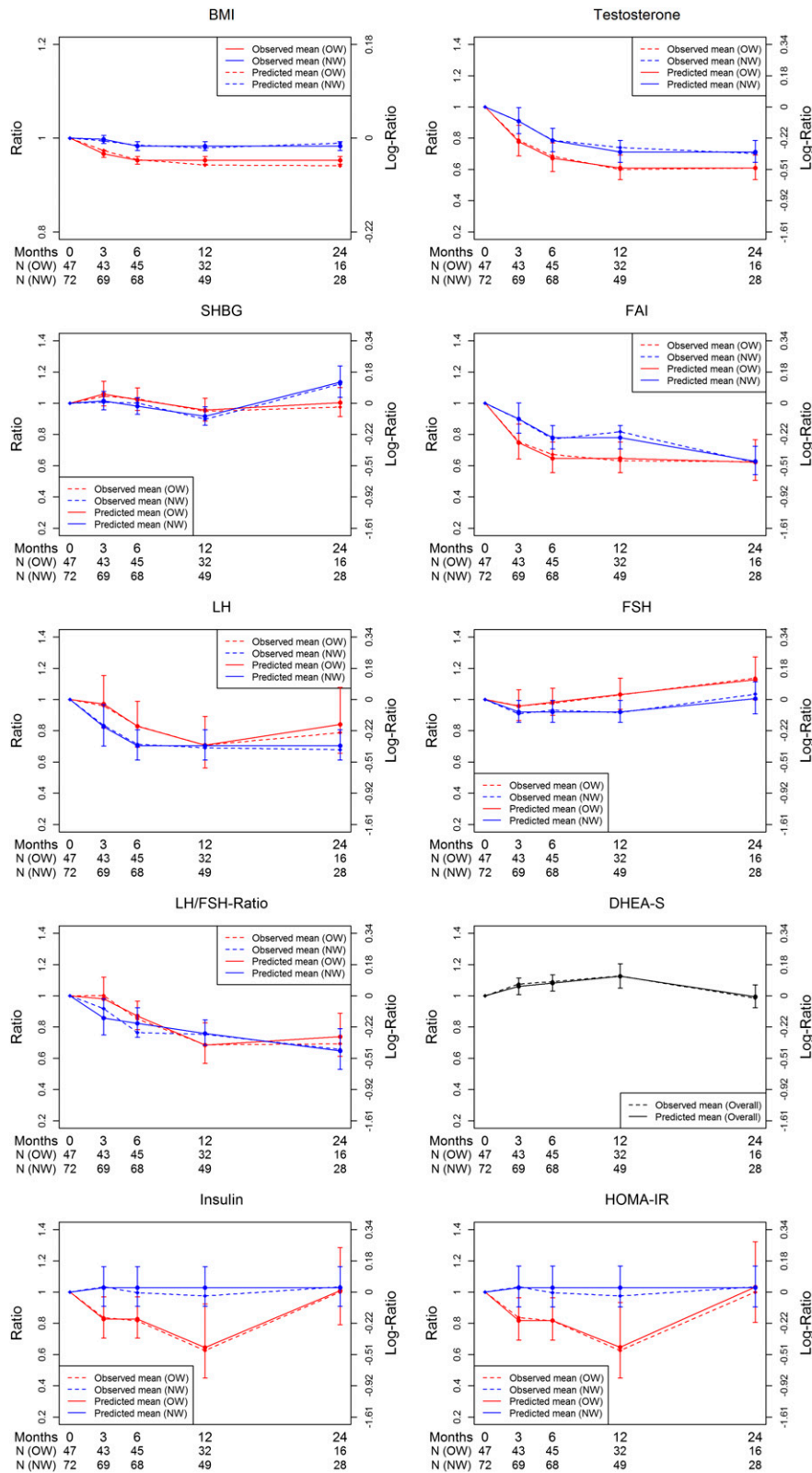
menstrual regularity, although the NW-ET subgroup was associated with a lower proportion of normal menses at baseline, all four BMI/testosterone subgroups achieved similar rates of normal menses at M6. The NW-ET subgroup was associated with the most significant improvement from the baseline, whereas the OW-NT subgroup was associated with the longest duration of improvement, as well as the highest rate of normal menses after treatment. The association between baseline BMI/testosterone phenotype and response in menstrual pattern can be useful in clinical prognostics.

The etiology for this difference in menstrual response between BMI/testosterone phenotypes is unknown, but probably relates to the different pathophysiological mechanisms displayed over the spectrum of clinical manifestations in PCOS. Although insulin resistance is a prominent feature of PCOS, it is heterogeneously present in women with PCOS (34), and it tends to manifest in obese patients (20). In contrast, lean women are more often associated with markers of gonadotropin dysfunction, such as elevated LH, and LH/FSH ratios (34). Although the culpable defect in PCOS remains undetermined, anovulation may result from a combination of hyperinsulinemia-associated hyperandrogenism (35) and LH-FSH-related ovulatory dysfunction (36). Both pathways have been linked to metformin.

In lean women with PCOS, metformin has reduced LH levels (37), whereas reductions in insulin resistance are seen in obese women (38). Although these findings were reported



**Figure 2.** Predicted and observed proportion of patients with normal menstrual cycles at each time point in a combined BMI and testosterone stratified analysis. NW is defined as BMI <25 kg/m<sup>2</sup>, OW as BMI ≥25 kg/m<sup>2</sup>, NT as serum testosterone <0.7 ng/mL, and ET as serum testosterone ≥0.7 ng/mL. N indicates sample size at each follow-up time.



**Figure 3.** Predicted and observed ratios of the mean measurement at each time point to the baseline measurement and the 95% CIs. NW is defined as BMI <25 kg/m<sup>2</sup>, and OW is defined as BMI ≥25 kg/m<sup>2</sup>. N (subgroup) indicates sample size at each follow-up time.

separately and attributed by some researchers to differences in study design rather than phenotypic differences (39), both effects were seen in our study. Additionally, although reductions in LH and testosterone were seen in

both subgroups, the change in LH was greater in the lean subgroup than in the obese subgroup. This was reversed for testosterone. The variable reductions in LH and testosterone across the different phenotypes may be the

**Table 4. Relative Changes in Value of Clinical Variables During Treatment in Relationship to the Baseline Are Presented as Ratios With Corresponding 95% CIs, as Calculated From the GEE Models**

Characteristics	Subgroup	M0	M3	M6	M12	M24
BMI	NW	1	1.00 [0.99, 1.01]	0.98 <sup>a</sup> [0.97, 0.99]	0.98 <sup>a</sup> [0.97, 0.99]	0.98 <sup>a</sup> [0.97, 0.99]
	OW	1	0.97 <sup>a</sup> [0.96, 0.97]	0.95 <sup>a</sup> [0.95, 0.96]	0.95 <sup>a</sup> [0.95, 0.96]	0.95 <sup>a</sup> [0.95, 0.96]
Testosterone	NW	1	0.91 <sup>a</sup> [0.83, 1.00]	0.79 <sup>a</sup> [0.71, 0.86]	0.71 <sup>a</sup> [0.64, 0.79]	0.71 <sup>a</sup> [0.64, 0.79]
	OW	1	0.78 <sup>a</sup> [0.69, 0.88]	0.67 <sup>a</sup> [0.58, 0.77]	0.61 <sup>a</sup> [0.54, 0.69]	0.61 <sup>a</sup> [0.54, 0.69]
SHBG	NW	1	1.02 [0.96, 1.07]	0.98 [0.93, 1.04]	0.92 <sup>a</sup> [0.86, 0.98]	1.13 <sup>a</sup> [1.04, 1.24]
	OW	1	1.06 [0.98, 1.14]	1.02 [0.95, 1.10]	0.96 [0.89, 1.03]	1.00 [0.91, 1.10]
FAI	NW	1	0.90 [0.81, 1.00]	0.78 <sup>a</sup> [0.71, 0.86]	0.78 <sup>a</sup> [0.71, 0.86]	0.63 <sup>a</sup> [0.54, 0.73]
	OW	1	0.75 <sup>a</sup> [0.64, 0.87]	0.65 <sup>a</sup> [0.56, 0.75]	0.65 <sup>a</sup> [0.56, 0.75]	0.62 <sup>a</sup> [0.51, 0.77]
LH	NW	1	0.83 <sup>a</sup> [0.70, 0.97]	0.70 <sup>a</sup> [0.61, 0.81]	0.70 <sup>a</sup> [0.61, 0.81]	0.70 <sup>a</sup> [0.61, 0.81]
	OW	1	0.97 [0.82, 1.15]	0.83 <sup>a</sup> [0.69, 0.99]	0.71 <sup>a</sup> [0.56, 0.89]	0.84 [0.66, 1.08]
FSH	NW	1	0.92 <sup>a</sup> [0.85, 0.99]	0.92 <sup>a</sup> [0.85, 0.99]	0.92 <sup>a</sup> [0.85, 0.99]	1.01 [0.91, 1.11]
	OW	1	0.96 [0.87, 1.05]	0.98 [0.90, 1.07]	1.03 [0.94, 1.13]	1.13 <sup>a</sup> [1.00, 1.27]
LH/FSH	NW	1	0.86 <sup>a</sup> [0.75, 0.98]	0.82 <sup>a</sup> [0.73, 0.93]	0.76 <sup>a</sup> [0.68, 0.85]	0.65 <sup>a</sup> [0.53, 0.79]
	OW	1	0.98 [0.86, 1.12]	0.87 <sup>a</sup> [0.79, 0.97]	0.69 <sup>a</sup> [0.57, 0.83]	0.74 <sup>a</sup> [0.61, 0.89]
DHEA-S	NW	1	1.06 <sup>a</sup> [1.01, 1.11]	1.08 <sup>a</sup> [1.03, 1.13]	1.12 <sup>a</sup> [1.05, 1.20]	1.00 [0.92, 1.07]
	OW					
Insulin	NW	1	1.03 [0.91, 1.16]	1.03 [0.91, 1.16]	1.03 [0.91, 1.16]	1.03 [0.91, 1.16]
	OW	1	0.83 <sup>a</sup> [0.71, 0.97]	0.83 <sup>a</sup> [0.71, 0.97]	0.65 <sup>a</sup> [0.45, 0.93]	1.01 [0.79, 1.29]
HOMA-IR	NW	1	1.03 [0.91, 1.17]	1.03 [0.91, 1.17]	1.03 [0.91, 1.17]	1.03 [0.91, 1.17]
	OW	1	0.82 <sup>a</sup> [0.69, 0.96]	0.82 <sup>a</sup> [0.69, 0.96]	0.65 <sup>a</sup> [0.45, 0.93]	1.03 [0.81, 1.32]

NW is defined as BMI <25 kg/m<sup>2</sup>, and OW is defined as BMI ≥25 kg/m<sup>2</sup>.

<sup>a</sup>*P* < 0.05.

basis for the variable response in menstrual patterns. However, this is a conjecture only, as the relative contributions of hyperandrogenism and LH/FSH imbalance on menstrual regularity across the spectrum of PCOS phenotypes are currently unknown.

Taken together, there are still gaps in our understanding of the mechanisms through which metformin affects women with PCOS, particularly in relationship to the changes in LH. Hypothetically, the effects of metformin are derived from its insulin-sensitizing activities (6), but the relationship between insulin metabolism and LH level remains controversial. Although insulin potentiates the gonadotropin-releasing hormone-elicited LH response in rat pituitary cells, this effect is not seen *in vivo* in humans (40). Furthermore, the suppressive effects of metformin on LH are often reported in non-insulin-resistant groups rather than insulin-resistant groups (41). Whether the effects of metformin on LH result from insulin-dependent or insulin-independent pathways remains uncertain (40), but much less is known about latter. Increased promoter activities of the gonadotrophin genes in the pituitary (42) and reduced androgen synthesis in ovarian theca cells have been seen in direct response to metformin (43) and could be the basis for the decreased levels in LH. Further work is needed to delineate this relationship.

One strength of the present study is the stratification of patients by BMI and testosterone phenotypes in the comparison of menstrual response, which has not been reported before. Although BMI and testosterone

have been considered separately as prognostic markers in the treatment of PCOS (20, 44), the use of BMI in combination with testosterone in the evaluation of treatment response to metformin is novel. Testosterone is a determinant of menstrual regularity (35), and stratification by both factors provides information pertaining to the interactions between these characteristics. Another strength of the present study is the longitudinal design, the repeated measurements obtained during follow-up, and the novel use of GEE models that adjusts for within-subject variations in repeated measurements while assessing for the effects of BMI/androgen phenotype on each of the outcome variables. This provides valuable information regarding how each clinical parameter changes in relationship to the duration of treatment. For instance, a time frame of particular importance in our study was the first 6 months of metformin use. This period was associated with most of the significant changes in BMI, testosterone, and LH levels. Significant changes in outcome measurements were rare after the first 6 months and were observed only for DHEA-S, SHBG, insulin, and HOMA in the OW subgroup, and for menstrual regularity in the OW-ET subgroup.

A limitation of this study is the lack of a control arm. As we were interested in the long-term effects of metformin, this study was designed as an observational study due to ethical concerns about the long-term use of placebos. Another point of concern is the high rate of dropouts and the effects it may have on the outcome of



the study. The percentages of patients that completed follow-up at M3, M6, M12, and M24 were 94%, 95%, 68%, and 37%, respectively. Although most patients completed follow-up visits for at least 6 months, the percentages of patients who did so were relatively low at 12 and 24 months. However, this attrition rate is similar to those from other studies on PCOS (13), and it is higher than that in the general population (45). Attempts were made to limit and assess the effects of dropouts.

The segmented regression analysis utilizing the GEE methodology evaluated all data in the longitudinal dataset and has been shown to be less susceptible to the effects of missing data (46). Additionally, we compared the dropout patients to the non-dropout patients using the logistic regression model, which tested all baseline characteristics as predictor of non-dropout status (Supplemental Table 4). No baseline characteristic was associated with non-dropout status. We also tested the consistency of the results by repeating the GEE segmented regression analysis solely on the 44 non-dropout patients. The results were generally compatible with the results from all 119 patients (Supplemental Table 5).

In conclusion, our study shows that metformin is associated with improvements in menstrual cycle regularity and most hormonal parameters in both OW and NW women with PCOS treated with metformin during the course of 24 months. In general, most clinical parameters reached maximal response after 6 months and subsequently maintained a steady-state. Variations in the pattern of improvements in the menstrual, hormonal, and metabolic profiles were seen among patients with different baseline BMI and testosterone phenotypes. This can serve as patient selection criteria or prognostics for metformin use.

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