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Metformin-diet benefits in women with polycystic ovary syndrome in the bottom and top quintiles for insulin resistance☆

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Abstract

We prospectively assessed whether metabolic and menstrual benefits of metformin-diet were equally realized in women with polycystic ovary syndrome (PCOS), categorized by pretreatment top (n = 32) and bottom (n = 35) quintile homeostasis model assessment insulin resistance (IR). Effects of metformin (2.55 g/d) and diet (6300-8400 J/d [1500-2000 cal/d], 26% protein, 44% carbohydrate) were prospectively assessed for 12 months. Pretreatment, the bottom and top insulin-resistant quintile groups differed by median weight (84 vs 121 kg), insulin (7.8 vs 40.5μU/mL), IR (1.62 vs 9.28), homeostasis model assessment insulin secretion (131 vs 416), glucose (82 vs 98 mg/dL), sex hormone–binding globulin (40 vs 15 nmol/L), (all $P < .0001$), free androgen index (2.76 vs 10.8) ($P < .001$), triglyceride (92 vs 131 mg/dL), high-density lipoprotein (46 vs 39 mg/dL), systolic blood pressure (116 vs 128 mm Hg), and diastolic blood pressure (76 vs 84 mm Hg), (all $P < .01$). After 12 months on metformin-diet, weight fell by 7% in both insulin-resistant groups ($P < .0001$), insulin, IR, and insulin secretion fell in the top insulin-resistant group by 60%, 64%, and 39% (all $P < .0001$), with smaller reductions in the bottom insulin-resistant group of 18%, 13% ($P > .05$ for both), and 22% ($P < .01$), respectively. The free androgen index fell 39% ($P > .01$) in the top insulin-resistant group. The pretreatment percentage of expected menses in the top insulin-resistant quintile (26 ± 39%) was 1.6 times less than in the bottom insulin-resistant quintile (41 ± 38%) ($P = .026$). Over the 12-month treatment period, the percentage of spontaneous regular normal menses increased to 72 ± 27% in the top insulin-resistant quintile group ($P < .0001$) and to 77 ± 31% in the bottom quintile group ($P < .0001$), with no group difference ($P = .33$). Metformin-diet metabolic effects were much more marked in women in the top vs the bottom quintile for IR. Women with PCOS in the bottom insulin-resistant quintile, conventionally thought not to respond optimally to metformin-diet, nevertheless experience significant metabolic and menstrual benefits. Metformin-diet should benefit most women with PCOS, even those with normal serum insulin, without IR.

1. Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy of women of childbearing age, affecting approximately 5% to 10% of women [1,2]. Polycystic ovary syndrome is characterized by oligomenorrhea, clinical and/or biochemical hyperandrogenism, and by polycystic ovaries [2-6]. Most, but not all women with PCOS, have fasting hyperinsulinemia with insulin resistance (IR) and high levels of insulin secretion (IS) [5,6]. Insulin-resistant patients with PCOS are heavier, more hirsute, have higher testosterone and lower sex hormone–binding globulin (SHBG) levels than non–insulin-resistant patients with PCOS [6].

Nestler and Jakubowicz [7] have examined whether hyperinsulinemia plays a role in the pathogenesis of PCOS in normal-weight or thin women. They [7] concluded, "hyperinsulinemia stimulates ovarian P450c17 alpha activity in non-obese women with PCOS" and that ". . . decreasing serum insulin with metformin reduces ovarian cytochrome P450c17 alpha activity and ameliorates the hyperandrogenism of these women." Acien et al [6] studied 137 women with PCOS and 75 without PCOS, and reported ". . . slim women with PCOS had insulin and metabolic variables similar to those without PCOS," and that most obese women with PCOS were insulin resistant and more hyperandrogenemic and hypertriglyceridemic than slim women with PCOS.

☆ This work was carried out with signed informed consent following a protocol approved by the Jewish Hospital Institutional Review Board.

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Acien et al [6] concluded "... insulin, androgens, and BMI are related in women both with and without PCOS, especially in obese ones." Toprak et al [8] used a euglycemic hyperinsulinemic clamp method to study 12 non-obese patients with PCOS and 10 healthy controls matched for age and weight. They reported that there was a significant degree of IR in non-obese patients with PCOS, and that their IR was related to serum LH and free testosterone levels. Gambineri et al [9] reported that, "... obese PCOS women have more severe hyperandrogenism and related clinical features (hirsutism, menstrual abnormalities, anovulation) than normal-weight PCOS women."

Although hyperinsulinemia and IR are neither necessary nor sufficient to produce PCOS [2-6,9,10], it has been our clinical experience [3,10,11] that physicians are reluctant to use metformin or other insulin-sensitizing agents in women with well-defined PCOS who have "normal" insulin levels. In 67 women with well-documented PCOS [4], categorized by pretreatment top (n = 32) and bottom (n = 35) quintile homeostasis model assessment (HOMA) IR [12], our specific aim was to assess whether metabolic and menstrual benefits of metformin-diet treatment differed between IR categories and whether women in the bottom insulin-resistant quintile experience significant metabolic and menstrual benefits.

2. Materials and methods

2.1. Cases

We used a protocol approved by the Jewish Hospital Institutional Review Board. All patients gave signed informed consent. Procedures followed were in accordance with the ethical standards for human experimentation established by the Declaration of Helsinki.

The diagnosis of PCOS (Tables 1 and 2) was made on the basis of the revised 2003 Rotterdam ESHRE/ASRM consensus criteria [4], with cases meeting 2 of the following 3 criteria after exclusion of other pathologies (pituitary insufficiency, persistent hyperprolactinemia, congenital adrenal hyperplasia, etc):

1. oligomenorrhea or anovulation;
2. clinical and/or biochemical signs of hyperandrogenism;
3. Polycystic ovaries.

Additional exclusion criteria in our study were serum creatinine of more than 1.5 mg/dL, type 1 diabetes mellitus, type 2 diabetes mellitus on pharmacologic therapy, less than 1 year of follow-up on metformin-diet, pregnancies in less than 1 year of treatment, and age less than 18 and older than 50 years.

We used the definitions of Laven et al [13] for oligomenorrhea (bleeding intervals between 35 days and 6 months) or amenorrhea (bleeding interval of more than 6 months) (Tables 1 and 2).

The percentage of occurrence of expected menses was calculated as number of menses over the number of expected menses. To provide the most complete and stable estimate of the pretreatment, study entry percentage of occurrence of expected menses, we used 1 year's previous menstrual history (Tables 1 and 2).

Table 1
Diagnostic characteristics of PCOS in 35 women with bottom-quintile HOMA IR at pretreatment study entry

	Number of menses in previous year					All n = 35
	=0	1-3	4-6	7-10	11-12	
	n = 8 (23%)	n = 11 (31%)	n = 6 (17%)	n = 4 (11%)	n = 6 (17%)	
Ferriman and Gallwey [39] scores ≥7	6 (75%)	10 (91%)	6 (100%)	4 (100%)	5 (83%)	31 (89%)
Severe acne	7 (88%)	2 (18%)	5 (83%)	2 (50%)	3 (50%)	19 (54%)
Clinical hyperandrogenism (FG ≥7 and/or severe acne)	8 (100%)	10 (91%)	6 (100%)	4 (100%)	6 (100%)	34 (97%)
Total testosterone >70 ng/dL[a]	2 (25%)	2 (18%)	1 (17%)	1 (25%)	1 (17%)	7 (20%)
Free testosterone >6.8 pg/mL[a]	1 (13%)	3 (27%)	1 (17%)	1 (25%)	1 (17%)	7 (20%)
Androstenedione >270 ng/dL[a]	3 (38%)	3 (27%)	1 (17%)	1 (25%)	1 (17%)	9 (26%)
DHEAS >270 μg/dL[a]	2 (25%)	1 (9%)	1 (17%)	2 (50%)	1 (17%)	7 (20%)
Biochemical hyperandrogenism (≥1 high androgen)	4 (50%)	4 (36%)	2 (33%)	2 (50%)	1 (17%)	13 (37%)
Clinical and/or biochemical hyperandrogenism	8 (100%)	10 (91%)	6 (100%)	4 (100%)	6 (100%)	34 (97%)
Polycystic ovaries confirmed	5 (63%)	7 (64%)	3 (50%)	2 (50%)	6 (100%)	23[b] (66%)
PCOS diagnosis [4], 2 of the 3: oligo-anovulation; clinical and/or biochemical hyperandrogenism; polycystic ovaries	8 (100%)	11 (100%)	6 (100%)	4 (100%)	6 (100%)	35 (100%)

DHEAS indicates dehydroepiandrosterone sulfate.

[a] 97.5th percentile.

[b] Pelvic ultrasound-laparotomy not done in the other 12 women.

Table 2
Diagnostic characteristics of PCOS in 32 women with top-quintile HOMA IR at pretreatment study entry

	Number of menses in previous year					All n = 32
	=0	1-3	4-6	7-10	11-12	
	n = 19 (59%)	n = 4 (13%)	n = 2 (6%)	n = 2 (6%)	n = 5 (16%)	
Ferriman and Gallwey [39] scores ≥7	16 (84%)	4 (100%)	2 (100%)	2 (100%)	4 (80%)	28 (88%)
Severe acne	9 (47%)	2 (50%)	2 (100%)	1 (50%)	2 (50%)	16 (52%)
Clinical hyperandrogenism (FG ≥7 and/or severe acne)	18 (95%)	4 (100%)	2 (100%)	2 (100%)	4 (80%)	30 (94%)
Total testosterone >70 ng/dL[a]	6 (32%)	1 (25%)	0 (0%)	1 (50%)	0 (0%)	8 (25%)
Free testosterone >6.8 pg/mL[a]	1 (5%)	0 (0%)	0 (0%)	1 (50%)	0 (0%)	2 (6%)
Androstenedione >270 ng/dL[a]	6 (32%)	1 (25%)	0 (0%)	2 (100%)	0 (0%)	9 (28%)
DHEAS >270 μg/dL[a]	2 (11%)	0 (0%)	0 (0%)	0 (0%)	2 (40%)	4 (13%)
Biochemical hyperandrogenism (≥1 high androgen)	8 (42%)	2 (50%)	0 (0%)	2 (100%)	2 (40%)	14 (44%)
Clinical and/or biochemical hyperandrogenism	19 (100%)	4 (100%)	2 (100%)	2 (100%)	5 (100%)	32 (100%)
Polycystic ovaries confirmed	18 (95%)	3 (75%)	2 (100%)	2 (100%)	5 (100%)	30[b](94%)
PCOS diagnosis [4], 2 of the 3: oligo-anovulation; clinical and/or biochemical hyperandrogenism; polycystic ovaries	19 (100%)	4 (100%)	2 (100%)	2 (100%)	5 (100%)	32 (100%)

[a] 97.5th percentile.
[b] Pelvic ultrasound-laparotomy shown negative in one woman and not done in the other woman.

2.2. Study protocol

At study entry, women with body mass index (BMI) of less than 25 or 25 kg/m^2 or higher [14] were, respectively, instructed in a 8400 or 6300 J/d (2000 or 1500 cal/d), high-protein (26% of energy [calories]), low-carbohydrate (44%) diet (42% of carbohydrate was complex), with 30% of the energy (calories) as fat and a polyunsaturated/saturated fat ratio of 2:1 [10,11]. Metformin was started at study entry, and the goal was to gradually increase the dose over a 1-month period to 2.55 g/d (850 mg 3 times per day with meals) as tolerated. No women used estrogen-progestin or progestin contraceptives during the 12-month follow-up period.

After their pretreatment baseline visit, women were seen every 2 to 3 months for outpatient follow-up visits of 1 year or more. At each outpatient visit, weight was measured and blood was obtained after an overnight fast for measurement of serum insulin, glucose, testosterone, SHBG, estradiol, progesterone, and plasminogen activator inhibitor activity [10,11,15]. The free androgen index (FAI) was calculated (testosterone × 3.467 / SHBG). At each visit, after a 5-minute resting period, seated blood pressure [16] was obtained by a single observer, and adherence to diet and to metformin was reviewed.

2.3. Statistical methods

As summarized in Figs. 1-4, within each insulin-resistant [12] quintile group we used paired Wilcoxon tests [17] to assess changes on metformin-diet for 4, 8, and 12 months from pretreatment baseline. Then, between insulin-resistant groups, we compared group median values by Wilcoxon tests at baseline, and at 4, 8, and 12 months on metformin-diet (Figs. 1-4). The percent changes displayed in Figs. 1-4 were the median of the percent changes in the variables studied.

Because paired comparisons were made within the 2 insulin-resistant quintile groups between baseline levels and levels at 4, 8, and 12 months on metformin-diet (Figs. 1-4), the method of Benjamini and Hochberg [18] was used to control for the false discovery rate. Because 4 comparisons were made for each variable between the 2 insulin-resistant quintile groups at pretreatment baseline and levels at 4, 8, and 12 months (44 comparisons, Figs. 1-4), the method of Benjamini and Hochberg [18] was further used to control for the false discovery rate. Only those P values that remained significant after controlling for the false discovery rate [18] are displayed in Figs. 1-5.

The Benjamini and Hochberg [18] approach to problems of multiple significance testing calls for controlling the expectation of the proportion of falsely rejected null hypotheses—the false discovery rate. This error rate is equivalent to the family-wise error rate when all null hypotheses are true, but is smaller otherwise, providing potential for a gain in power [18].

Pretreatment vs on metformin-diet menstrual frequency (% menses), calculated using data from 12 months of pretreatment and 12 months of metformin-diet, were compared within top and bottom insulin-resistant quintile groups using paired Wilcoxon tests [17] (Fig. 5). To compare the % menses between top and bottom insulin-resistant quintile groups, pretreatment and on 12 months of metformin-diet therapy, Wilcoxon tests [17] were used (Fig. 5).

In an attempt to assess whether the benefits observed were due to the insulin-sensitizing effect of metformin or to the weight loss, a subgroup analysis was done to examine

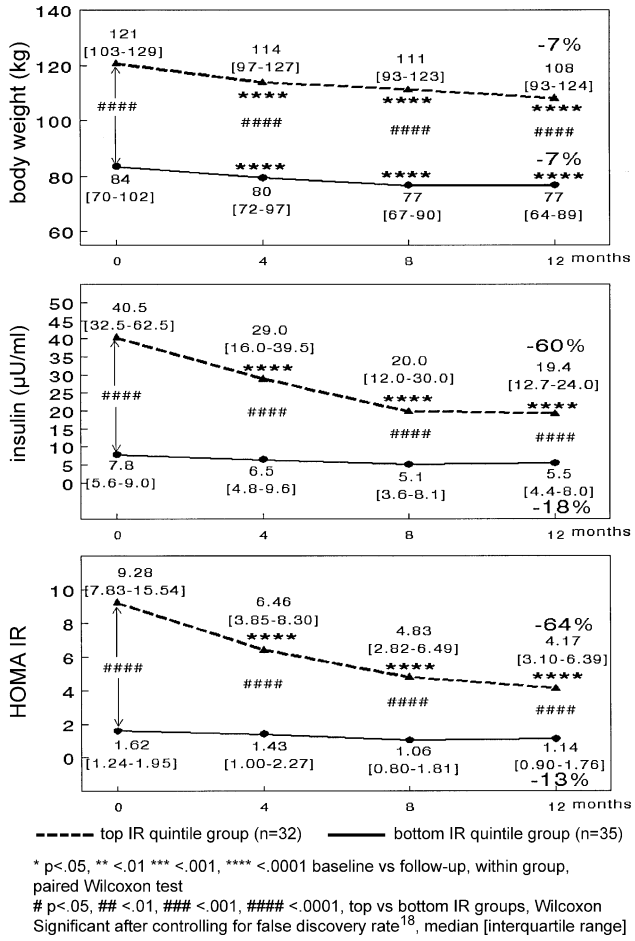

Fig. 1. Median and interquartile range of body weight, insulin, and HOMA IR [12] at pretreatment baseline, and after 4, 8, and 12 months on metformin-diet in 35 women in the bottom insulin-resistant quintile group and 32 women in the top insulin-resistant quintile group. The median of percent change from pretreatment baseline to levels at 12 months is displayed for both quintile groups.

the metabolic and menstrual changes in the women who did not experience weight loss, defined as weight loss of 1% or less at 12 months of follow-up.

3. Results

3.1. Universe of patients with PCOS

Over an 8.5-year period (July 17, 1995-January 28, 2004), of 1065 women referred for diagnosis and therapy for PCOS, 898 were characterized as having PCOS by the revised 2003 Rotterdam consensus criteria [4].

After obtaining pretreatment, baseline measures, all 898 women were started on metformin-diet. Of these 898 women, 15 were pregnant at referral, leaving 883, of whom 80 conceived in 1 year or less of follow-up, leaving 803 women. Of these 803 women, 531 have had less than 1-year of follow-up, and 272 had 1 year or more of follow-up, of whom 249 were 18 to 50 years old. Of these 249 women, 7 could not conceive, 140 did not wish to conceive, and 102 wished to conceive.

The 249 women were categorized by their pretreatment HOMA IR [12] into quintiles. In the 49 cases in the bottom insulin-resistant quintile, median IR was 1.47, with a range of 0.04 to 2.04. In the 50 cases in the top insulin-resistant quintile, median IR was 9.19, with a range of 7.01 to 74.7. Of the 50 women in the top quintile, complete follow-up data for 3 periods (<5, 5 to <9, 9 to <14 months) were available in 32; of the 49 women in the bottom quintile, 35 had complete data (Tables 1 and 2). To be included in the analyses, the women were required to have complete data for the variables displayed in Tables 1 and 2 and in Figs. 1-5.

Of the 32 women in the top insulin-resistant quintile, 6 (19%) took 1500 to 1700 mg metformin per day and 26 (81%) took 2500 to 2550 mg/d. Of the 35 women in the bottom insulin-resistant quintile, 7 (20%) took 1500 to 1700 mg metformin per day, 5 (14%) took 2000 to 2200 mg/d, and

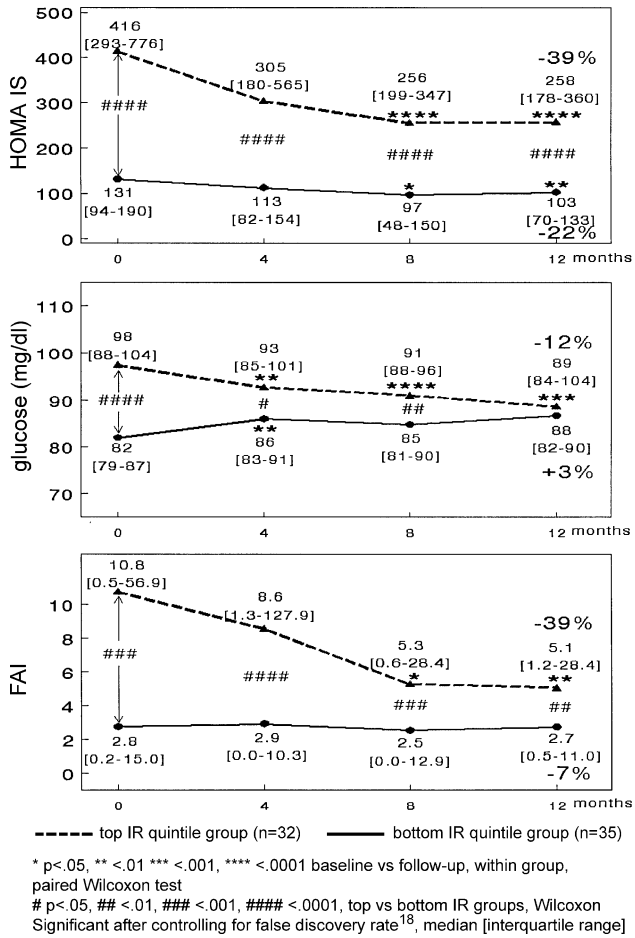

Fig. 2. Median and interquartile range of HOMA IS [12], glucose, and FAI at pretreatment baseline, and after 4, 8, and 12 months on metformin-diet in 35 women in the bottom insulin-resistant quintile group and 32 women in the top insulin-resistant quintile group. The median of percent change from pretreatment baseline to levels at 12 months is displayed for both quintile groups.

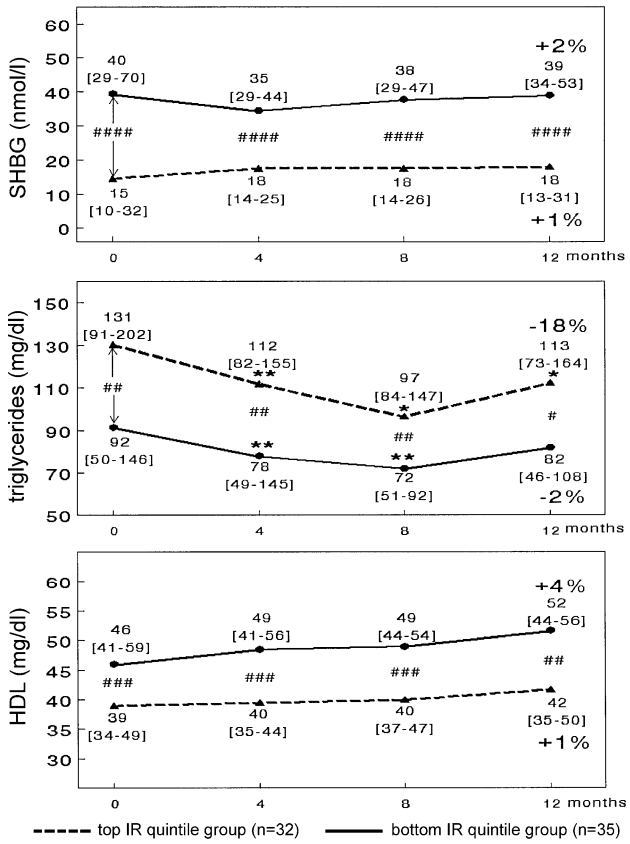

Fig. 3. Median and interquartile range of SHBG, triglycerides, and HDL-C at pretreatment baseline, and after 4, 8, and 12 months on metformin-diet in 35 women in the bottom insulin-resistant quintile group and 32 women in the top insulin-resistant quintile group. The median of percent change from pretreatment baseline to levels at 12 months is displayed for both quintile groups.

23 (66%) took 2550 mg/d. Because we did not obtain serial written 72-hour dietary records at each outpatient visit, we cannot quantitatively characterize dietary adherence.

3.2. Characteristics of PCOS in the 35 and 32 women in pretreatment bottom and top insulin-resistant quintiles

As summarized in Tables 1 and 2, by selection, all women in the study met the 2003 consensus criteria [4] for the diagnosis of PCOS.

Median ages at study entry were 32 for women in the bottom insulin-resistant quintile and 32 in the top quintile (P = .76). Pretreatment women in the top insulin-resistant quintile were heavier (121 vs 84 kg), had higher fasting serum insulin (40.5 vs 7.8 μU/mL) and glucose (98 vs 82 mg/dL), higher HOMA IR (by selection) (9.28 vs 1.62), and higher HOMA IS (416 vs 131) (Figs. 1 and 2). Women in the top insulin-resistant quintile had lower SHBG (15 vs 40 nmol/L) (P < .0001) and high-density lipoprotein cholesterol (HDL-C) (39 vs 46 mg/dL) (P < .001), higher FAI (10.8 vs 2.8, P < .001), higher triglycerides (131 vs 92

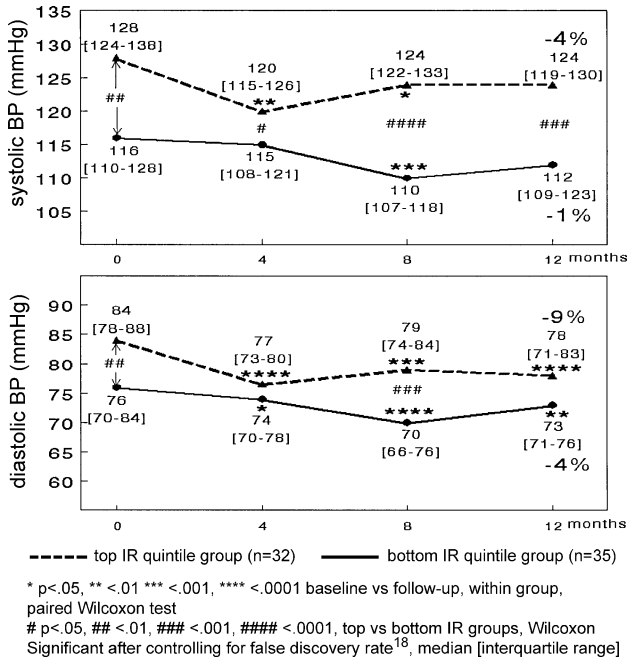

Fig. 4. Median systolic and diastolic blood pressure at pretreatment baseline, and after 4, 8, and 12 months on metformin-diet in 35 women in the bottom insulin-resistant quintile group and 32 women in the top insulin-resistant quintile group. The median of percent change from pretreatment baseline to levels at 12 months is displayed for both quintile groups.

mg/dL) (P = .01), and higher systolic (128 vs 116 mm Hg) and diastolic blood pressure (84 vs 76 mm Hg) (P < . 01) (Figs. 1-4).

In the top insulin-resistant quintile group, no women had normal weight (BMI < 25), and none were overweight (BMI ≥ 25-30) [14], 41% were obese (BMI ≥ 30-40) [14], and 59% had extreme obesity (BMI ≥ 40) [14]. Although thinner than the women in the top insulin-resistant quintile,

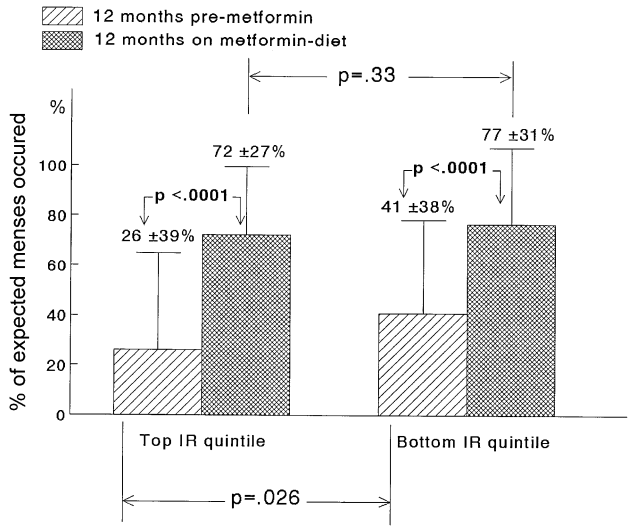

Fig. 5. Pre-metformin vs on-metformin-diet menstrual frequency (mean ± SD of % expected menses that occurred), calculated using data from 12 months of pretreatment and from 12 months on metformin-diet in 35 women in the bottom insulin-resistant quintile group and 32 in the top insulin-resistant quintile.

of the 35 women in the bottom insulin-resistant quintile, only 29% had normal weight, 11% were overweight, 54% were obese, and 6% had extreme obesity [14]. The BMI distribution was shifted toward obesity and extreme obesity in the top insulin-resistant quintile group vs the bottom insulin-resistant group ($\chi^2 = 28.8$, $df = 3$, $P < .0001$).

3.3. Longitudinal changes in weight, insulin, HOMA IR, HOMA IS, glucose, SHBG, FAI, triglycerides, HDL-C, systolic and diastolic blood pressure

Controlling for the false discovery rate [18], in the top insulin-resistant group over 12 months of treatment, the reductions in weight, insulin, IR, IS, glucose, FAI, triglycerides, and systolic and diastolic blood pressure remained significant (Figs. 1-4). In the bottom insulin-resistant group, controlling for the false discovery rate [18], significant changes included reductions in weight (Fig. 1), HOMA IS (Fig. 2), and systolic and diastolic blood pressure (Fig. 4).

Over the 12-month follow-up period, both quintile groups lost a median 7% of body weight (Fig. 1). Throughout follow-up, the top-quintile insulin-resistant group had higher body weight (Fig. 1).

Fasting serum insulin fell in both quintile groups, with the median reduction being 60% at month 12 in the top insulin-resistant group vs 18% in the bottom insulin-resistant group (Fig. 1). Median insulin levels were higher throughout follow-up in the top-quintile insulin-resistant group (Fig. 1). Insulin resistance fell by 64% in the top insulin-resistant quintile group and fell by 13% in the bottom insulin-resistant group (Fig. 1). Homeostasis model assessment IS fell on metformin-diet, being 39% lower at 12 months than at pretreatment baseline in the top insulin-resistant group and 22% lower in the bottom insulin-resistant group (Fig. 2). Insulin secretion levels were higher throughout follow-up in the top-quintile insulin-resistant group (Fig. 2).

Glucose fell 12% in the top insulin-resistant quintile group, without significant change in the bottom insulin-resistant quintile (Fig. 2).

The FAI fell at months 8 and 12 in the top-quintile insulin-resistant group (Fig. 2). The FAI was 39% lower at 12 months of follow-up than at pretreatment baseline in the top-quintile insulin-resistant group ($P < .01$, Fig. 2). Conversely, there were no significant reductions in FAI in the bottom-quintile insulin-resistant group at 12 months of follow-up (Fig. 2).

High-density lipoprotein and SHBG were not significantly altered by metformin-diet in either insulin-resistant quintile groups (Fig. 3). Throughout follow-up, the top-quintile insulin-resistant group had lower SHBG and lower high-density lipoprotein (Fig. 3).

Systolic and diastolic blood pressure both fell over time in both quintile groups, with more pronounced changes in the top insulin-resistant vs the bottom insulin-resistant group, with larger decrements in diastolic vs systolic blood pressure (Fig. 4).

In the top-quintile insulin-resistant group, triglycerides fell 18% on metformin-diet, with the top-quintile insulin-resistant group maintaining higher triglyceride levels throughout therapy (Fig. 3).

Subgroup analysis could not be done within the top and bottom insulin-resistant quintile groups in women who did not experience weight loss (≤1% weight loss at 12 months of follow-up) because of very small numbers, 2 of 35 women in the bottom quintile and 2 of 32 in the top quintile.

3.4. Longitudinal changes in menstrual status

The pretreatment percentage of expected menses in the top insulin-resistant quintile (26 ± 39%) was 1.6 times less than in the bottom insulin-resistant quintile (41 ± 38%) ($P = .026$). Over the 12-month treatment period, the percentage of spontaneous regular normal menses increased to 72 ± 27% in the top insulin-resistant quintile group ($P < .0001$) and to 77 ± 31% in the bottom quintile group ($P < .0001$) with no group difference ($P = .33$) (Fig. 5).

4. Discussion

The women in the current study came from a large cohort with well-defined PCOS, all treated with diet-metformin, irrespective of pretreatment insulin and IR. Beyond exclusion of women who conceived in the first year of follow-up, and the requirement for complete data for the variables in the current analysis, the current study group is representative of women with well-defined [4] PCOS referred to our center for treatment with diet and insulin-sensitizing drugs, predominantly metformin.

An optimal assessment of metformin-diet effects in women with PCOS having top and bottom quintile pretreatment IR would prospectively randomize women to diet-placebo and diet-metformin groups, allowing independent assessment of the effects of diet and diet-metformin. The current prospective study, which incorporated concurrent diet-metformin, cannot separate effects of diet alone vs diet-metformin. Because only 2 of 35 women in the bottom quintile and 2 of 32 in the top quintile had weight loss of 1% or less after 12 months of therapy, there were insufficient subjects to allow subgroup analysis of metformin benefit independent of weight loss.

It is possible that some of the benefits observed in the current study were attributable to the fact that the diet was low in carbohydrates [19-21]. We [19] have previously used metformin (2.55 g) and a diet of 6300 J (1500 cal) (26% protein, 44% carbohydrate [42% of carbohydrate complex], 30% fat [polyunsaturated/saturated ratio 2:1]) in 64 women with both PCOS and the metabolic syndrome. After 6 months of therapy, mean body weight fell 4.7%, triglycerides 14%, systolic blood pressure 5%, diastolic blood pressure 6%, and insulin 31%. Farnsworth et al [20]

compared a high-protein diet (27% of energy [calories] as protein, 44% as carbohydrate, 29% as fat) with a standard protein diet (16% protein, 57% carbohydrate, 27% fat) during 12 weeks of energy restriction and 4 weeks of energy balance in 57 overweight hyperinsulinemic volunteers. Although weight loss did not differ between the 2 groups, glycemic response fell more in the high protein diet group. In 132 obese adults, Stern et al [21] restricted carbohydrate to less than 30 g/d (low-carbohydrate diet) or restricted energy (caloric) intake by 2100 J (500 cal/d) with less than 30% of energy fraction (calories) from fat (conventional diet). There were more favorable metabolic responses to the low-carbohydrate diet after adjustment for weight loss differences [21].

Serum insulin, androgens, and BMI are related in women both with and without PCOS [6]. Acien et al [6] noted that "... slim women with PCOS had insulin and metabolic variables similar to those without PCOS ...." Although the women in the bottom insulin-resistant quintile of the current study had normal fasting serum insulin and IR, with IR well below cut-off values of 2.5 [12], and were much thinner, had lower FAI and higher SHBG than those in the top insulin-resistant quintile, as a group they were not slim. Pretreatment women in the bottom insulin-resistant quintile had mean BMI of 30.9 with 11% overweight (BMI $\geq$ 25-30), 54% obese (BMI $\geq$ 30-40), and 6% extremely obese (BMI $\geq$ 40) [14]. Median weight reduction on metformin-diet for 12 months in the 35 women in the bottom insulin-resistant quintile (7%) was the same as in the 32 women in the top insulin-resistant quintile (7%), a major benefit of metformin-diet. Weight loss in both insulin-resistant quintile groups may have been sufficiently high [22-24] to explain the observed improvements in menstrual status and to have augmented effects of metformin [3,10,11] on insulin, IR, IS, and FAI.

Insulin sensitivity is a continuous variable and there is no consensus on a lower limit cut-off point where the declining insulin sensitivity could be called IR [25]. Fasting serum insulin has been used as a surrogate index of insulin sensitivity [10,11,26], but explains only 30% to 40% of the variance in glucose-clamp determined insulin sensitivity, which is the gold standard [27]. The HOMA model [12] that we used in our study can be reliably used in large-scale or epidemiologic studies in which only a fasting blood sample is available to assess insulin sensitivity [26]. Although fasting serum insulin is a simple, practical, and inexpensive correlate of IR [10,11,26], it is a weak link on which to base diagnosis and treatment decisions in PCOS, which, as in the current study, should be based on the diagnostic oligomenorrhea, clinical-biochemical hyperandrogenism, and/or polycystic ovaries [2,4,28].

In the current study, in women in the top insulin-resistant quintile vs those in the bottom quintile, metformin-diet had a more substantive effect on fasting serum insulin, IR, IS, glucose, FAI, triglycerides, and systolic and diastolic blood pressure. However, metformin-diet significantly increased menstrual frequency and regularity in a similar fashion irrespective of pretreatment insulin-resistant quintile group, such that after 12 months on therapy, menstrual frequency did not differ by insulin-resistant quintile, despite having been 1.6 times lower at pretreatment baseline in women in the top insulin-resistant quintile. A potential source of bias in both quintile groups is that the pre-metformin menstrual frequencies were based on the recall of the past year by the subjects, and thus subject to recall bias, whereas the post-metformin menstrual frequencies were monitored during the clinical trial. We speculate that there is considerable variation in "local-ovarian" IR or relative IR, such that metformin can more directly affect ovarian function [29-31] than the other endocrine abnormalities of PCOS.

Using cultured ovarian granulosa cells, Wu et al [29] reported that "... there were significant decreases in insulin-stimulated glucose incorporation into glycogen in PCOS cells, which is a metabolic action of insulin." Troglitazone increased insulin-induced glycogen synthesis in cultured ovarian granulosa cells [29]. Wu et al [29] concluded "... there is a selective defect in insulin actions in PCOS granulosa cells which suggests ovarian insulin resistance." "Troglitazone could divergently alter expression of various IRS molecules and insulin actions and could be used as an ovarian insulin sensitizer ..." [29]. Attia et al [30] treated human ovarian theca-like tumor cells in vitro with various concentrations of metformin and reported that metformin had a direct effect on the androgen production of thecal cell. In cultured thecal cells, in vitro, Mansfield et al [31] reported that metformin inhibited androstenedione production in a dose-dependent fashion. Hence, as in the current report, the insulin-sensitizing effect of metformin may have a direct ovarian effect [7], whereas serum insulin and IR are not significantly changed in women with PCOS in the bottom insulin-resistant quintile.

Insulin and LH stimulate de novo androgen biosynthesis in primary cultures of porcine thecal cells [32] Troglitazone dose-dependently antagonizes LH-insulin's combined stimulation of androstenedione and testosterone by thecal cells in vitro [32]. This offers a plausible mechanistic basis for the clinical efficacy of troglitazone and metformin in reducing androgen excess in women with PCOS [32]. In PCOS, high levels of androgenic hormones interfere with the pituitary-ovarian axis, leading to increased LH levels, anovulation, amenorrhea, and infertility [33,34].

Morin-Papunen et al [35] recently studied 17 non-obese women with PCOS (BMI < 25), comparing metformin (1 g/d for 3 months, then 2 g/d for 3 months) with ethinyl estradiol cyproterone acetate. Metformin improved hyperandrogenism, hyperinsulinemia, and menstrual cyclicity, "... most likely through its positive effect on insulin clearance and abdominal obesity." Morin-Papunen et al [35] concluded "... thus, similarly to obese PCOS women, non-obese PCOS subjects with anovulation may also benefit from metformin treatment." A second major benefit of metformin in both insulin-resistant and non–insulin-resistant women with

PCOS may be its ability to reduce C-reactive protein [36] and endothelin-1 [37]. Morin-Papunen et al [36] reported that metformin (1-2 g/d) decreased serum C-reactive protein levels, particularly in obese subjects with PCOS. Diamanti-Kandarakis et al [37] reported that obese and non-obese women with PCOS had higher levels of endothelin-1 than controls, and that endothelin-1 was reduced significantly in both obese and non-obese subjects with PCOS by metformin (1700 mg/d). Metformin also improved hormonal and metabolic profiles in both obese and non-obese subjects [37]. Because fasting serum insulin is an independent risk factor for coronary heart disease events [38], reduction of metformin in insulin and IR should be anti-atherogenic [36,37].

To our knowledge, this is the first study that has assessed response to metformin-diet in both uniformly insulin-resistant and insulin-sensitive women with PCOS. In the current study, although metabolic benefits of metformin therapy were much more marked in women in the top than the bottom insulin-resistant quintile, probably related to major differences between groups at pretreatment baseline, both groups had comparable improvement in menstrual regularity and weight loss. Hence, metformin-diet offers significant benefit not only to severely insulin-resistant women with PCOS, but also to women with PCOS in the bottom quintile for IR. We conclude that metformin-diet should not be withheld from treatment of PCOS in women with normal fasting serum insulin and no evidence for IR.

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