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Combined Low-Dose Pioglitazone, Flutamide, and Metformin for Women with Androgen Excess

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Context and Objective: One of the treatments for hyperinsulinemic hyperandrogenism in nonobese women is combined androgen receptor blockade (with flutamide; Flu), insulin sensitization (with metformin; Met) plus an estroprogestagen contraceptive. We tested whether adding low-dose pioglitazone (Pio; 7.5 mg/d) confers more benefit.

Setting: The study was conducted at a university hospital.

Study Population and Design: This double-blind study enrolled 38 young women with hyperinsulinemic hyperandrogenism [mean body mass index (BMI) 24 kg/m^2], all of whom started on Flu (62.5 mg/d) and Met (850 mg/d) plus a transdermal estroprogestagen, each for 21 of 28 d over 6 months. Patients were randomly assigned to receive, in addition, placebo (n=19) or Pio (n=19; 7.5 mg/d) for the same 21 of 28 d over 6 months.

Main Outcomes: BMI, waist to hip ratio, hirsutism score, fasting endocrine-metabolic markers, body composition, abdominal fat (visceral *vs.* sc), and carotid intima-media thickness were measured at study start and after 6 months.

Results: PioFluMet reduced intima-media thickness more than FluMet and lowered glucose, IGF-I, and C-reactive protein more as well as the ratio of low-density lipoprotein to high-density lipoprotein cholesterol and the ratio of neutrophils to lymphocytes. PioFluMet treatment was followed by a leaner body composition and a loss of visceral fat (both $P < 0.001$). In the total group, the changes included not only decreases in waist to hip ratio, hirsutism score, and testosterone (all $P < 0.001$) but also minor drops in alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transpeptidase, and lactate dehydrogenase (all $P < 0.005$), indicating absence of hepatotoxicity; BMI remained unchanged. Clinical side effects were not detected.

Conclusion: In this proof-of-concept study, addition of Pio to FluMet plus an estroprogestagen led to improvements in the endocrine-metabolic condition, in low-grade inflammation, in total and visceral adiposity, and in markers of cardiovascular health. **(*J Clin Endocrinol Metab* 92: 1710–1714, 2007)**

ONE OF THE treatments for hyperinsulinemic ovarian hyperandrogenism is combined androgen receptor blockade (with flutamide; Flu), insulin-sensitization (with metformin; Met), and, if needed, lifestyle changes and contraceptive measures (1–5).

Peroxisome proliferator activated receptor-γ agonists [thiazolidinediones (TZDs)] are a novel class of insulin-sensitizing agents that are used in the treatment of type 2 diabetes but have also been shown to improve the endocrine state and ovulatory performance of women with androgen excess (6–8). TZDs inhibit peripheral lipolysis and have antiinflammatory, antioxidant, and antiprocoagulant properties (6–9). The clinical use of TZDs is limited by potential side effects including weight gain, which is mostly due to edema and/or gain of fat mass; these side effects are partly dose and host dependent (10).

Here we explored whether the addition of the TZD pioglitazone (Pio) in low dose (7.5 mg/d; commonly used doses are 4- to 6-fold higher) added benefit to the effects of low-dose flutamide-metformin (FluMet) in women with androgen excess.

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Abbreviations: ALT, Alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein; CV, coefficient of variation; DHEAS, dehydroepiandrosteronesulfate; Flu, flutamide; γ-GT, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; IMT, intima-media thickness; LDL, low-density lipoprotein; Met, metformin; MRI, magnetic resonance imaging; Pio, pioglitazone; SAT, sc adipose tissue; TZD, thiazolidinedione; VAT, visceral adipose tissue; WHR, waist to hip ratio.

Subjects and Methods

Study population

In this proof-of-concept study, the population consisted of 38 young women with hyperinsulinemic hyperandrogenism [mean ± SEM; age 19.6 ± 0.3 yr; range 18–24 yr; body mass index (BMI) 23.7 ± 0.5 kg/m^2, range 19.5–29.0 kg/m^2; 5–12 yr after menarche; Table 1].

Inclusion criteria were hyperinsulinemia on a standard 2-h oral glucose tolerance test, defined as peak serum insulin levels greater than 150 U/ml and/or mean serum insulin greater than 84 μU/ml (11, 12); and ovarian androgen excess, as defined by: 1) hirsutism [Ferriman-Gallwey score > 8 (13)], amenorrhea (menses absent for more than 3 months), or oligomenorrhea (menstrual cycles > 45 d); 2) high serum androstenedione, total testosterone, or free androgen index [testosterone × 100/SHBG] (12); and 3) a 17-hydroxyprogesterone hyperresponse (>160 ng/dl) to a GnRH agonist (leuprolide acetate 500 μg sc) (12, 14).

Before study start, none of the patients were receiving a contraceptive

TABLE 1. Clinical, endocrine-metabolic, carotid ultrasound, body composition (by absorptiometry), and abdominal MRI indices in young women with androgen excess who received treatment with low-dose FluMet (Flu, 62.5 mg/d; Met, 850 mg/d) and a transdermal estroprogestagen and who were randomized to receive in addition either placebo (21 of 28 d; n = 19) or low-dose Pio (7.5 mg/d; 21 of 28 d; n = 19) for 6 months

	All	Placebo			Pio		
	At 0 months	0 months[a]	6 months	Δ 0–6 months	0 months[a]	6 months	Δ 0–6 months
Age (yr)	**19.6 ± 0.3**	19.2 ± 0.3	19.7 ± 0.3	**0.5 ± 0.0**	19.9 ± 0.5	20.4 ± 0.3	**0.5 ± 0.0**
BMI (kg/m^2)	**23.7 ± 0.5**	23.1 ± 0.6	23.0 ± 0.6	**−0.1 ± 0.2**	24.3 ± 0.6	24.5 ± 0.6	**0.2 ± 0.2**
Fasting insulin (mU/liter)	**11.9 ± 0.8**	10.9 ± 1.2	9.5 ± 1	**−1.5 ± 1.1**	12.8 ± 1	11.1 ± 0.9	**−1.8 ± 1.1**
Fasting glucose (mg/dl)	**94 ± 1**	91 ± 1	89 ± 1	**−2 ± 1**	96 ± 1	90 ± 1[b]	**−7 ± 2**[c]
IGF-I (ng/ml)	**269 ± 10**	273 ± 14	183 ± 13[b]	**−89 ± 10**	266 ± 15	136 ± 10[b]	**−130 ± 14**[c]
LDL to HDL ratio	**2.1 ± 0.1**	2.1 ± 0.1	1.9 ± 0.1[d]	**−0.2 ± 0.1**	2.2 ± 0.1	1.7 ± 0.1[e]	**−0.5 ± 0.1**[c]
Testosterone (ng/dl)	**83 ± 3**	82 ± 5	51 ± 5[b]	**−30 ± 6**	84 ± 5	50 ± 5[b]	**−34 ± 5**
SHBG (nmol/liter)	**36 ± 2**	37 ± 3	167 ± 9[b]	**130 ± 8**	35 ± 3	174 ± 8[b]	**139 ± 8**
Androstenedione (ng/dl)	**464 ± 24**	455 ± 33	330 ± 26[b]	**−126 ± 24**	472 ± 37	287 ± 23[b]	**−185 ± 33**
DHEAS (μg/dl)	**264 ± 18**	264 ± 22	196 ± 19[b]	**−69 ± 13**	263 ± 29	213 ± 25[d]	**−50 ± 20**
ALT (U/liter)	**15.1 ± 0.8**	14.3 ± 1.1	12.1 ± 0.7[d]	**−2.2 ± 1**	15.8 ± 1.3	12.8 ± 1[d]	**−3.0 ± 1.4**
Hematocrit (%)	**40.1 ± 0.4**	40.0 ± 0.4	38.4 ± 0.4[b]	**−1.5 ± 0.3**	40.3 ± 0.6	37.7 ± 0.6[b]	**−2.6 ± 0.5**
Neutro to lympho ratio	**1.9 ± 0.1**	1.7 ± 0.1	1.5 ± 0.1	**−0.2 ± 0.1**	2.1 ± 0.1	1.6 ± 0.1[b]	**−0.5 ± 0.1**[c]
CRP (mg/liter)	**3.7 ± 0.3**	3.8 ± 0.2	2.6 ± 0.3[b]	**−1.1 ± 0.3**	3.7 ± 0.5	1.9 ± 0.3[b]	**−1.8 ± 0.5**[c]
IMT (mm)[f]	**0.47 ± 0.01**	0.46 ± 0.01	0.40 ± 0.02[b]	**−0.06 ± 0.01**	0.48 ± 0.02	0.36 ± 0.02[b]	**−0.12 ± 0.02**[c]
Total lean mass (kg)	**35.1 ± 0.7**	35.0 ± 0.7	35.5 ± 0.8	**0.4 ± 0.2**	35.1 ± 1.1	36.0 ± 1.2[b]	**0.9 ± 0.1**
Total fat mass (kg)	**22.6 ± 1.1**	21.9 ± 1.7	22.2 ± 1.7	**0.3 ± 0.5**	23.3 ± 1.5	22.5 ± 1.4[d]	**−0.8 ± 0.3**
Body fat fraction (%)	**38.5 ± 1.2**	37.6 ± 1.9	37.7 ± 1.7	**0.1 ± 0.7**	39.3 ± 1.4	38.0 ± 1.3[b]	**−1.4 ± 0.3**[c]
L3 visc fat (cm^2)	**53 ± 3**	51 ± 3	48 ± 3	**−3.4 ± 3.1**	54 ± 5	46 ± 4[b]	**−7.8 ± 1.4**
L3 sc fat (cm^2)	**163 ± 12**	153 ± 18	158 ± 19	**4.9 ± 6.7**	172 ± 16	176 ± 16	**4.0 ± 3.9**

Values are mean ± SEM. Indicative values from 24 asymptomatic young women are: testosterone, 31 ± 3 ng/dl; androstenedione, 156 ± 14 ng/dl; DHEAS, 125 ± 12 μg/dl (26). To convert units to SI, multiply the concentrations of testosterone by 0.03467, those of androstenedione by 0.0349, and those of DHEAS by 0.02714; divide the concentrations of SHBG by 0.0288, those of triglycerides by 88.5, and those of HDL cholesterol and LDL cholesterol by 38.7. Neutro, Neutrophil count; lympho, lymphocyte count; visc, visceral; L3, lumbar vertebra 3 level. *Boldface* highlights the main variables at baseline and the differences at 6 months in favor of Pio.

[a] Absence of significant differences between randomized subgroups at 0 months.

[b] $P \leq 0.001$ *vs.* baseline (0 months).

[c] $P < 0.05$ for 0- to 6-month change (Δ) *vs.* placebo.

[d] $P < 0.05$ *vs.* baseline (0 months).

[e] $P \leq 0.01$ *vs.* baseline (0 months).

[f] Indicative values from 16 asymptomatic young women: 0.39 ± 0.02 mm (19).

or another medication known to affect gonadal or adrenal function, or carbohydrate or lipid metabolism, for at least 9 months.

Exclusion criteria were evidence of thyroid dysfunction, Cushing's syndrome or hyperprolactinemia; glucose intolerance (15); personal history of diabetes mellitus; late-onset adrenal hyperplasia (16, 17); anemia; abnormal serum electrolytes; abnormal screening results for liver or kidney function; or abnormal echocardiogram.

This study was registered as ISRCTN12871246 and conducted in Barcelona, without support from industry, after approval by the Institutional Review Board of Sant Joan University Hospital and after informed consent by each of the patients. None of the results in the present manuscript has been reported previously.

Study design

In this double-blind, placebo-controlled study, all women started on metformin (850 mg/d) and Flu (62.5 mg) once daily (21 of 28 d), at dinnertime, and a transdermal contraceptive with ethinylestradiol 600 μg plus norelgestromin 6 mg, via a weekly patch (Evra, 21 of 28 d; Janssen-Cilag, Beerse, Belgium) for 6 months. After stratification for BMI, patients were randomly assigned [1:1 ratio, Gran Mos program, Barcelona Medical Research Institute (12, 18)] to receive, in addition, placebo (21 of 28 d; n = 19) or Pio (7.5 mg, 21 of 28 d; n = 19), at breakfast time, for 6 months. FluMet and Pio/placebo were all discontinued (7 of 28 d) during the cyclic week off contraception.

The randomization sequence was known only to a pharmacist independent to the study and was thus unknown to the clinically involved investigators. Pio and placebo were packaged in similar tablets; renewals were scheduled thrice monthly. All patients and investigators, except for the study statistician (A.L.-B.), have so far been, and still are, blinded for the treatment allocations.

Clinical and endocrine-metabolic variables, carotid intima-media thickness (IMT), body composition, and abdominal fat distribution were all assessed at study start (0 months) and again after 6 months during the off-treatment week.

Clinical assessment

Height (by Harpenden stadiometer), weight, BMI [ratio of weight (in kilograms) to height squared (in meters)], waist to hip ratio (WHR), and hirsutism score (13) were in each subject assessed by the same investigator (L.I., blinded to treatment allocation).

Endocrine-metabolic assessment

Fasting blood glucose, neutrophil and lymphocyte count, serum insulin, low-density lipoprotein (LDL)- and high-density lipoprotein (HDL)-cholesterol, SHBG, testosterone, dehydroepiandrosteronesulfate (DHEAS), C-reactive protein (CRP), and IGF-I were measured together with alanine- and aspartate aminotransferase (ALT and AST, respectively), γ-glutamyl transpeptidase (γ-GT), and a screening of renal function.

Baseline assessments were performed in the follicular phase (d 3–7) or after 2 months of amenorrhea.

Carotid IMT

Longitudinal ultrasound scans of the carotid arteries were obtained by the same investigator (G.E., blinded to treatment allocation) who used a high-resolution apparatus with color and power Doppler capabilities (Acuson Sequoia 512 SHA; Medisales, Los Alamitos, CA) and a high-frequency 10-MHz linear probe (19). The right and left common carotid

arteries and the bifurcation-bulb areas were scanned in multiple planes. Images were obtained from the distal portions of both common carotid arteries, 1–2 cm away from the bulb and immediately proximal to the origin of the bifurcation. The IMT of the posterior (far) wall of both common carotid arteries was measured as the distance between the junction of the lumen and intima and the junction of the media and adventitia (19, 20). IMT was on each side recorded as the mean of five measures. The intraobserver coefficient of variation (CV) was less than 10%. In line with a previous paper on IMT in women with androgen excess (19), we report IMT results of the left carotid, the slice volumes of which are similar to those on the right side (21).

Body composition

Body composition was assessed by dual-energy x-ray absorptiometry with a Lunar Prodigy and Lunar software (version 3.4/3.5; Lunar Corp., Madison, WI) (12). Total irradiation dose per assessment was 0.1 mSievert; CVs for scanning precision are 2.2 and 2.6% for fat and lean mass, respectively (22).

Assessment of abdominal fat distribution

Total sc (SAT) and visceral adipose tissue (VAT) areas were measured by magnetic resonance imaging (MRI) using a whole-body multislice MRI 1.5 Tesla device (Signa LX Echo Speed Plus Excite; General Electric Healthcare, Milwaukee, WI). Subjects were placed on the platform with arms extended above the head, according to standard imaging procedures (23). All patients were scanned using a T1-weighted spin-echo sequence with 360 msec repetition time, 21 msec echo time, 40 cm field of view, and 256 × 224 matrix. To obtain abdominal MRI fat values, transverse slices of 10-mm thickness were acquired beginning at the L4-L5 intervertebral space. SAT and VAT areas were measured by fitting a spline curve to points on the border of the sc and visceral regions, selected by the same operator (L.d.R., blinded to treatment allocation). Nonfat regions within the visceral region were also outlined with a spline fit and subtracted from the total visceral region. The visceral fat region was subdivided into retroperitoneal and intraperitoneal areas using the ascending and descending colon, the psoas muscles on each side of the spine, and the top of the vessels above the vertebrae as guides for the spline fit. VAT area was calculated by subtracting the organ areas from the intraperitoneal area (24). CVs for SAT and VAT were 7.2 and 8.8%, respectively. These CVs were obtained by repeating the scan three times within 6 months in 10 young women and were calculated by dividing the SE of the estimate from linear regression analysis by the mean of the measurements.

Assays and statistics

Neutrophil and lymphocyte counts were assessed by cell counter (ABX Pentra 120; ABX Diagnostics, Montpellier, France) (25). Serum glucose was measured by the glucose oxidase method. Immunoreactive insulin was assayed by microparticle enzyme immunoassay (Imx; Abbott Diagnostics, Santa Clara, CA); the mean intra- and interassay CVs were 4.7 and 7.2%, respectively. Serum testosterone, 17-hydroxyprogesterone hyperresponse, DHEAS, androstenedione, and SHBG were measured by immunochemiluminescence (IMMULITE 2000; Diagnostic Products, Los Angeles, CA). All methods had intra- and interassay CVs between 4 and 8% within the relevant concentration ranges. CRP was measured by a highly sensitive method (Architect c8000; Abbott, Wiesbaden, Germany) with intra- and interassay CVs less than 2%; the detection limit was 0.1 mg/liter. Serum samples were stored at −20 C until assay. Statistical analyses were performed with SPSS 12.0 (SPSS Inc., Chicago, IL). For uniformity, results are expressed as mean ± SEM. Non-Gaussian variables were mathematically transformed to improve symmetry before statistical analyses. Two-sided *t* tests were used for comparisons between groups, and for paired samples within groups; significance level was set at $P < 0.05$.

Results

Table 1 summarizes the results in both subgroups at baseline and after 6 months. The placebo subgroup confirmed the known effects of intermittent (21 of 28 d) therapy with low-dose FluMet plus a transdermal estroprogestagen (26), extended those findings over time (from 3 to 6 months), and disclosed that hematocrit and circulating IGF-I are lowered ($P < 0.001$), that serum ALT falls reassuringly ($P < 0.05$), and that carotid IMT is reduced ($P < 0.001$). None of the adiposity markers, however, changed detectably within 6 months.

Pio addition to FluMet was followed by additional benefits such as a leaner body composition and a loss of visceral fat (baseline *vs.* 6 months; $P < 0.001$ for both), a further reduction of IMT and body adiposity (PioFluMet *vs.* FluMet; $P < 0.05$ for 0- to 6-month changes in both markers). Other effects of Pio addition were a lowering of fasting glycemia and LDL to HDL ratio as well as further reductions of circulating IGF-I and inflammatory markers such as CRP and neutrophil to lymphocyte ratio (PioFluMet *vs.* FluMet; $P < 0.05$ for 0- to 6-month changes in all these indices), whereas the reassuring ALT drop was maintained (Fig. 1).

In the total study population, 0- to 6-month changes included not only decreases in insulin, glucose, IGF-I, visceral fat, WHR, hirsutism score, testosterone, LDL to HDL ratio, neutrophil to lymphocyte ratio, CRP, and IMT (Fig. 1) but also minor drops in serum ALT, AST, γ-GT, and lactate dehydrogenase (all $P <$

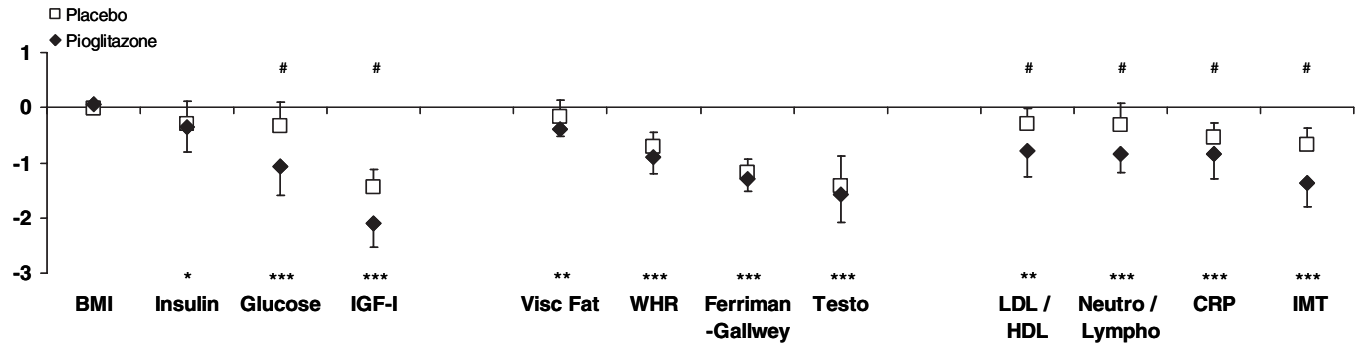

FIG. 1. Changes (0–6 months) in endocrine-metabolic indices, visceral fat, inflammation markers, and carotid IMT of young women with androgen excess treated with low-dose FluMet (21 of 28 d), a transdermal estroprogestagen (21 of 28 d), and either placebo or low-dose Pio (7.5 mg/d; 21 of 28 d). Changes are expressed as SD scores, calculated by dividing the individual values by the corresponding baseline SD in the study subjects. Plots represent means ± 95% confidence intervals. *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ for 0- to 6-month changes within the total group. #, $P < 0.05$ for differences in 0- to 6-month changes between subgroups. Visc Fat, Visceral fat section at lumbar vertebra 3 level; Testo, testosterone; Neutro, neutrophil count; Lympho, lymphocyte count.

0.005), indicating absence of hepatotoxicity. Clinical side effects were not detected.

Discussion

In women with androgen excess, the addition of low-dose Pio (21 of 28 d) to a combination of low-dose FluMet and a transdermal estroprogestagen (21 of 28 d) resulted in additional reductions of LDL to HDL ratio, CRP, neutrophil to lymphocyte ratio, and carotid IMT, which are markers of metabolic and cardiovascular health over the longer term. CRP promotes atherothrombosis through direct effects on endothelial cells and vascular smooth muscle cells, and neutrophils enhance coagulation in localized areas of inflammation, *i.e.* on injured endothelium or at sites of platelet aggregate deposition (27–29). Pio and other TZDs down-regulate the expression of CRP and other endothelial activation markers, reduce platelet activity in the circulation, and delay atheromatous plaque progression. The atheroprotective effects of TZDs, as reflected in a reduction of IMT, could thus result through not only insulin sensitization but also other pathways, *i.e.* via a direct TZD effect on the vessel wall or from a TZD-mediated fall in the LDL to HDL ratio (30, 31).

In hyperandrogenic women, Pio therapy (30 mg/d) is known to be associated with a robust weight gain that is mainly ascribed to gain of sc fat, which, in turn, seems to develop despite augmented GH secretion and despite maintained serum levels of IGF-I (32–34). Here we found that the addition of a much lower Pio dose to FluMet plus an estroprogestagen is in hyperandrogenic women followed by a paradoxal reduction of body adiposity and a further fall of circulating IGF-I. We speculate that Pio's dose-effect curves for insulin sensitization and nonvisceral adipogenesis are respectively shaped so that if Pio is given in low dose, its insulin-sensitizing effects prevail over its adipogenic effects. Nocturnal profiling of GH secretion may clarify whether low-dose Pio reduces circulating IGF-I here by acting as a partial GH antagonist (35) or rather as an IGF-I sensitizer. The mechanisms whereby cotherapy with low-dose Pio elicits additional benefits are likely to be multiple and intertwined, and they clearly require more research in women with androgen excess.

In line with other studies, we observed a small but consistent fall in hematocrit under low-dose FluMet treatment, either with or without Pio addition (36). These falls in hematocrit are thought to be mainly attributable to concomitant drops in circulating androgens and thus in androgen-driven hematopoiesis (37).

Women with androgen excess are at risk for transaminase elevations, which have been attributed to nonalcoholic steatohepatitis (38) or the hepatotoxicity of high-dose Flu (39). Both low-dose FluMet and low-dose PioFluMet were here reassuringly accompanied by minor but consistent drops in the serum levels of ALT, AST, γ-GT, and/or lactate dehydrogenase. It should be emphasized that the study design included several measures to reduce hepatic risk. First, Flu (62.5 mg/d) and Pio (7.5 mg/d) were each given in the lowest dose known to be effective; for both compounds, this low dose is associated with a virtual absence of side effects (4, 12, 18, 26, 40–42). Second, because the combination of Pio and Flu is unprecedented, the respective doses were daily given with a maximal intradiem interval (morning *vs.* evening). Third, to avoid a hepatic first-pass effect of orally ingested estroprogestagens, we used a transdermal contraceptive that was previously studied in a combination with FluMet (26). Finally, there was a complete medication-free week after every 3-wk episode on estroprogestagen plus either FluMet or PioFluMet.

The additive effects of low-dose Pio, as detected within 6 months in this relatively small study population, may be statistically subtle, but they were achieved on top of striking changes ($P < 0.001$ for IGF-I, CRP, and IMT) obtained in the placebo group, which actually received one of the most effective treatments known so far. For some indices, low-dose Pio amplified the benefits of Flu-Met (plus an estroprogestagen) by another approximately 50% to approximately 100%; integrated changes of such magnitude are unprecedented in young women with androgen excess.

In this proof-of-concept study, the addition of low-dose Pio to FluMet plus an estroprogestagen led to improvements in the endocrine-metabolic condition, low-grade inflammation, total and visceral adiposity, and markers of cardiovascular health. Larger trials of longer duration are warranted to assess the long-term efficacy and safety of low-dose PioFluMet therapy in women with androgen excess.

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