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Clinical Research Article

Ibuprofen Reduces Testosterone Level in Women With Polycystic Ovary Syndrome

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Abstract

Context: Hyperandrogenism is a central feature of polycystic ovary syndrome (PCOS). In vitro studies have demonstrated that inflammatory stimuli promote whereas ibuprofen inhibits androgen production by ovarian theca-interstitial cells.

Objective: This work aimed to determine the effects of nonselective inhibitor of cyclooxygenases COX-1 and COX-2 on testosterone levels.

Methods: A prospective pilot study took place in an academic hospital of women with PCOS defined according to Rotterdam criteria (N = 20). Evaluations were taken at baseline and after 3 weeks of ibuprofen administration (400 mg twice a day or 400 mg 3 times a day, respectively, in women with weight < and ≥ 70 kg). The main outcome measure was total serum testosterone.

Results: Ibuprofen administration was associated with a decline of total testosterone from 0.75 ± 0.06 ng/mL to 0.59 ± 0.05 ng/mL ($P = .008$). There was no statistically significant change in the levels of other relevant hormones including dehydroepiandrosterone sulfate, gonadotropins, and insulin. Multiple regression analysis identified the greatest decline of testosterone was independently predicted by baseline testosterone level ($P = .004$) and by baseline insulin sensitivity index ($P = .03$).

Conclusion: Nonselective inhibition of COX-1 and COX-2 leads to selective reduction of testosterone consistent with direct inhibitory effect on ovarian steroidogenesis.

Key Words: polycystic ovary syndrome, testosterone, insulin resistance

Abbreviations: BMI, body mass index; FSH, follicle-stimulating hormone; ISI, insulin sensitivity index; LH, luteinizing hormone; LPS, lipopolysaccharides; OGTT, oral glucose tolerance test; PCOS, polycystic ovary syndrome; PGE2, prostaglandin E_2; TSH, thyrotropin; WHR, waist-to-hip ratio.

Polycystic ovary syndrome (PCOS), the most common endocrine disorder among women of reproductive age, is associated with hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology [1-4]. While the pathophysiology of this syndrome is still poorly understood, the central feature of PCOS is excessive production of androgens by ovarian theca cells [5]. Over the last 2 decades, accumulated evidence has demonstrated that PCOS is associated with low-grade systemic inflammation characterized by increased concentration of leukocytes, C-reactive protein, and several proinflammatory cytokines [6-10]. Our recent studies have revealed that women with PCOS have elevated serum markers of endotoxemia: lipopolysaccharides (LPS) and LPS-binding protein [11], possibly due to increased gut wall permeability and/or altered gut microbiome [12].

In vitro experiments indicate that proinflammatory stimuli may contribute to increased synthesis of androgens; indeed, in studies of isolated rat theca-interstitial cells, we found that LPS and interleukin 1β directly stimulate androgen production by increasing expression of the key gene regulating androgen synthesis: *Cyp17a1* [13]. Furthermore, molecules with pronounced anti-inflammatory properties, such as statins and resveratrol, inhibit expression of *Cyp17a1* and reduce androgen production in theca-interstitial cells [14, 15]. In clinical trials, statins and resveratrol reduced testosterone levels in women with PCOS [16-20].

More recently we found that a nonsteroidal anti-inflammatory drug, ibuprofen, inhibited androgen production by rat theca-interstitial cells abrogating stimulatory actions of LPS and interleukin 1β [21]. These effects were observed at a pharmacologic concentration of ibuprofen (0.1 mM; human studies of individuals taking 600 mg of ibuprofen twice a day for 6 weeks [22, 23]).

In view of the aforementioned observations, we carried out a pilot trial in women with PCOS evaluating effects of ibuprofen on serum testosterone and other relevant hormones.

Materials and Methods

Participants

The study was carried out at the Reproductive Endocrinology & Infertility Clinical Services at Poznan University of Medical

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Sciences in Poland. All participants fulfilled PCOS criteria as defined by the Rotterdam consensus [24] and had at least 2 of the following: 1) clinical or laboratory evidence of hyperandrogenism; 2) oligomenorrhea or amenorrhea; and/or 3) polycystic ovaries as determined by transvaginal ultrasound [25]. Individuals with congenital adrenal hyperplasia, hyperprolactinemia, thyroid disease, Cushing disease, and diabetes mellitus were excluded. During the 2 months prior to the study, none of the study participants used any form of hormonal therapy such as contraceptive pills. Written informed consent was obtained from all participants. Approval of the study was obtained from the institutional review board at the Poznan University of Medical Sciences. The study was registered at www.clinicaltrials.gov with the identifier NCT04485403.

Procedures

The flowchart of this study is outlined in Fig. 1. All participants were evaluated at baseline and after 3 weeks of weeks of ibuprofen administration (400 mg twice a day in women with weight < 70 kg and 400 mg 3 times a day in women with weight ≥ 70 kg). This adjustment of daily doses according to weight was based on evidence that heavy individuals exhibit increased clearance of ibuprofen and require higher doses to achieve adequate plasma concentration [26]. Clinical assessments included determinations of body mass index (BMI), waist-to-hip ratio (WHR), and hirsutism (using Ferriman-Gallwey score). Transvaginal ultrasound evaluations were performed using a Voluson S8 Touch (General Electric Co). Ovarian volume was calculated using the prolate ellipsoid formula.

Venous blood was collected after an overnight fast. Serum specimens were stored at −70 °C until analysis was performed. A 2-hour oral glucose tolerance test (OGTT) was performed with determinations of glucose and insulin in the fasting state as well as after a 75-g glucose load at 60 and 120 minutes. Glucose was determined by hexokinase method using a Roche Cobas e6001 immunoanalyzer (Roche Polska sp z o.o.). Insulin, total testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), sex hormone–binding globulin, 17-hydroxyprogesterone, and dehydroepiandrosterone sulfate, were determined using specific electrochemiluminescence assays (Roche Cobas e6001 immunoanalyzer; Roche Polska sp z o.o.). The insulin sensitivity index (ISI) was calculated using glucose and insulin levels obtained during an OGTT as described by Matsuda and DeFronzo [27]: ISI = (10 000/square root of [(fasting glucose × fasting insulin) × (mean glucose × mean insulin during OGTT)].

Statistical Analysis

Analysis was carried out using JMP pro 15 statistical software (SAS Institute). *P* values less than .05 were considered statistically significant. Comparisons of baseline and

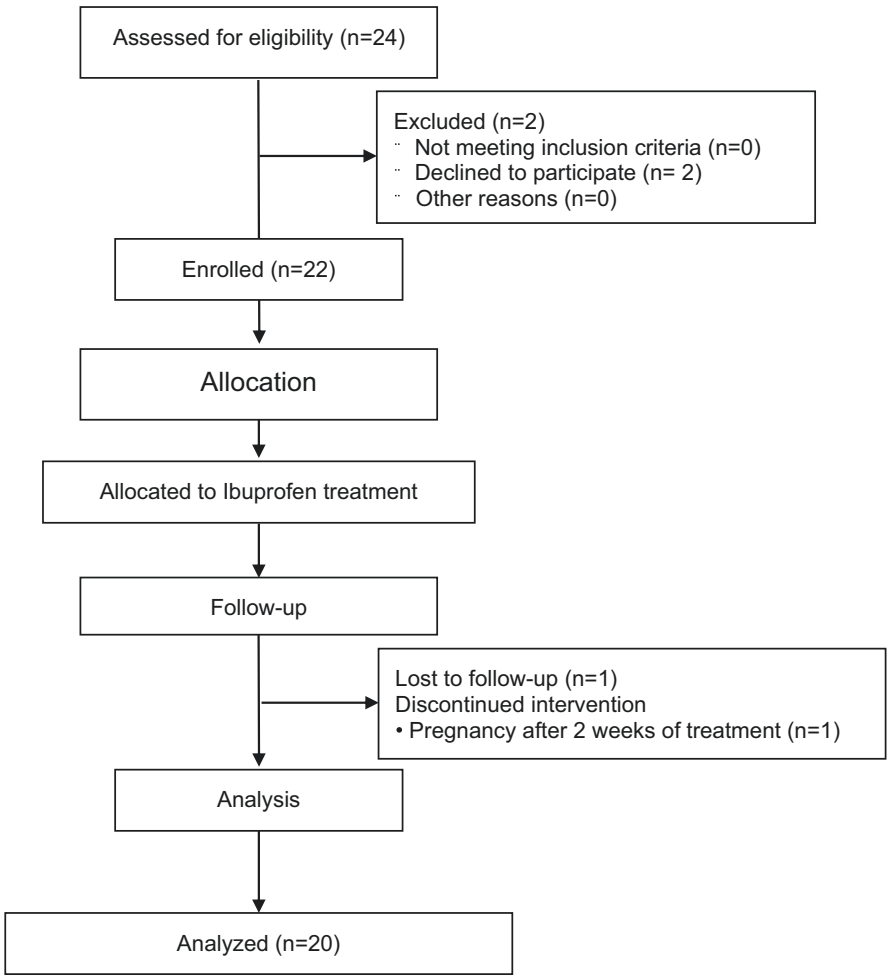

Figure 1. CONSORT flow diagram of the study.

follow-up values were performed using the paired *t* test. In the absence of a normal distribution (tested by Anderson-Darling test), Wilcoxon signed rank testing was carried out. Correlations between ordinal variables were performed using the Spearman rank test. Multiple regression modeling was performed using a backward stepwise approach. Power analysis revealed that the evaluation of 20 participants will have greater than 90% power to detect a 20% reduction of total testosterone with a coefficient of variation of 100%, and an α error of .05.

Results

The CONSORT flow diagram of the study (see Fig. 1) demonstrates that 20 individuals completed the 3-week trial, as planned at the time of registration of the study at clinicaltrials.gov. The average age and BMI of the participants were, respectively, 26.7 ± 0.8 years and 27.3 ± 1.1 (mean ± SEM). Before commencement of ibuprofen treatment, 95% (19/20) of participants who subsequently completed the trial had evidence of either clinical or biochemical hyperandrogenism: A total of 90% (18/20) had total testosterone greater than 0.5 ng/mL, and 80% (16/20) had hirsutism (Ferriman-Gallwey score ≥ 8).

Effects of Treatment

Table 1 presents levels of tested variables at baseline and at the end of the 3-week treatment with ibuprofen. The primary outcome, testosterone level, declined by 21 ± 7% (P = .008) in parallel with a 28 ± 11% decrease of free testosterone (P = .01). In contrast, there was no statistically significant change in levels of other tested hormones, including dehydroepiandrosterone sulfate, gonadotropins, and measures of insulin sensitivity.

Table 1. Baseline and posttreatment levels of endocrine and metabolic parameters in women with polycystic ovary syndrome

Variable	Baseline	Post ibuprofen	*P*
Total testosterone, ng/mL	0.75 ± 0.06	0.59 ± 0.05	**.008**
Free testosterone, ng/dL	1.22 ± 0.14	0.88 ± 0.09	**.01**
DHEAS, µmol/L	11.0 ± 1.1	10.2±1.0	.37
SHBG, nmol/L	43.8 ± 4.6	47.3 ± 4.7	.56
LH, mIU/mL	14.2 ± 2.5	13.0 ± 1.7	.99
FSH, mIU/mL	5.8 ± 0.3	5.8 ± 0.4	.90
LH to FSH ratio	2.46 ± 0.42	2.23 ± 0.26	.93
Estradiol, pg/mL	78.0 ± 11.7	67.6 ± 6.8	.95
Prolactin, µg/L	14.5 ± 1.4	12.5 ± 1.5	.24
TSH, mIU/L	2.5 ± 0.3	2.4 ± 0.6	.79
Insulin fasting, µU/mL	14.0 ± 3.0	13.5 ± 2.0	.76
AUC insulin	366 ± 50	352 ± 49	.67
Glucose fasting, mg/dL	94.7 ± 1.5	95.5 ± 1.4	.60
AUC glucose	498 ± 17	485 ± 20	.47
ISI	3.81 ± 0.52	3.49 ± 0.38	.47

Each value represents mean ± SEM; values in bold are statistically significant.
Abbreviations: AUC, area under the curve; DHEAS, dehydroepiandrosterone sulfate; FSH, follicle-stimulating hormone; ISI, insulin sensitivity index; LH, luteinizing hormone; SHBG, sex hormone–binding globulin; TSH, thyrotropin.

Analysis of the subgroups of women receiving lower doses of ibuprofen (400 mg twice a day) vs higher dose (400 mg 3 times a day) reveled a nonsignificant trend toward greater reduction in testosterone level in those receiving the lower dose of the medication (–0.25 ± 0.09 ng/mL; 29 ± 10%) vs the higher dose (–0.10 ± 0.07 ng/mL; 15 ± 10.0%) (P = .17). Women receiving lower doses of ibuprofen had statistically significantly lower BMI (P = .008).

Table 2 demonstrates that decline of testosterone correlated significantly with WHR, baseline levels of total testosterone, and free testosterone. Notably, since the dependent variable is defined as "decline," the most negative value, that is, the greatest decline of testosterone, correlated with lower WHR, higher total testosterone and higher free testosterone. Multiple regression analysis revealed the greatest decline of testosterone correlated independently with baseline testosterone level and baseline ISI; these 2 variables explained 50% of the variance (adjusted R^2) in this model (Table 3).

Discussion

The present study demonstrates that a short 3-week course of a nonsteroidal anti-inflammatory agent, ibuprofen, led to a statistically significant reduction of circulating testosterone level in women with PCOS. This observation sheds new light on the understanding of the mechanism regulating

Table 2. Correlations of clinical, endocrine, and metabolic parameters with change in total testosterone (univariate analysis)

Variable	Spearman ρ	*P*
Age	–0.12	.61
Age of menarche	0.20	.39
Menses per y	–0.09	.70
BMI	0.14	.55
WHR	0.52	**.018**
Hirsutism	–0.10	.67
Total ovarian volume	–0.05	.82
Total testosterone	–0.73	**<.001**
Free T, ng/dL	–0.48	**.04**
E_2, pg/mL	–0.05	.85
DHEAS, µmol/L	–0.006	.98
SHBG, nmol/L	–0.14	.59
LH, mIU/mL	–0.22	.36
FSH, mIU/mL	–0.23	.32
LH/FSH	–0.14	.54
Prolactin, µg/L	0.05	.84
TSH, mIU/L	0.33	.15
Insulin fasting, µU/mL	0.19	.43
AUC insulin	0.22	.36
Glucose fasting	0.03	.89
AUC glucose	–0.03	.90
ISI	–0.21	.36

Abbreviations: AUC, area under the curve; BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; E_2, estradiol; FSH, follicle-stimulating hormone; ISI, insulin sensitivity index; LH, luteinizing hormone; SHBG, sex hormone–binding globulin; TSH, thyrotropin; WHR, waist-to-hip ratio.

Table 3. Multiple linear regression model predicting decline of total testosterone level

Term	Estimate	t ratio	*P*
Intercept	0.40	3.07	.007
Testosterone baseline	–0.53	–3.37	.004
ISI	–0.04	–2.41	.03

Adjusted R-squared: 0.50.

androgen production and may provide the basis for a search for new therapeutic approaches aimed at control of hyperandrogenism. We are not aware of previous publications describing the effects of nonsteroidal anti-inflammatory on the reproductive aspects of female physiology; however, a previous study in men revealed that ibuprofen administration led to "compensated hypogonadism" [22]. In that study, LH and ibuprofen plasma levels were positively correlated and the ratio of testosterone to LH was reduced.

Current concepts describing the regulation of ovarian androgen production focus on direct actions of LH on ovarian theca cells resulting in upregulated expression of key enzymes, including Cyp11a1 and Cyp17a1 [28-30]. Another well-recognized endocrine stimulator of androgen synthesis is insulin either acting alone or in synergy with LH [31, 32]. In the present study, decline of testosterone level following ibuprofen treatment was not associated with statistically significant changes in LH or insulin levels, suggesting that ibuprofen actions are not mediated by these hormones and may be due to direct actions at the level of the ovary. Indeed, in our previous experiments on rat theca-interstitial cells, we found that ibuprofen profoundly reduces androgen production and inhibits RNA expression of Cyp11a1 and Cyp17a1 [21].

Ibuprofen is a nonselective inhibitor of cyclooxygenases COX1 and COX 2, the enzymes responsible for conversion of arachidonic acid to active prostaglandins, including proinflammatory prostaglandin E_2 (PGE2), an important paracrine mediator in the ovary. Indeed, granulosa cells of women with PCOS produce and release greater amounts of PGE2 than cells from women without PCOS [33]. In animal studies, PGE2 was shown to stimulate testosterone production [28]. In view of the aforementioned considerations and the present findings, we hypothesize that ibuprofen directly inhibits ovarian PGE2 production and hence reduces androgen synthesis. In view of the side effects of ibuprofen, its long-term use in hyperandrogenic patients cannot be recommended. Furthermore, since the process of ovulation involves the activation of inflammatory pathways [34], the administration of nonsteroidal anti-inflammatory drugs should be avoided in women desiring ovulation. However, the present findings point to the potential for novel treatments of excessive androgen production by the development of novel treatments targeting inflammation, or possibly selective inhibition of production and/or action of PGE2.

Another potentially clinically relevant observation is the relationship of ISI with the response to ibuprofen treatment (see Table 3), whereby women with the greatest insulin sensitivity experienced a greater decline of testosterone. In other words, those with insulin resistance, and hence compensatory hyperinsulinemia, were less likely to respond to ibuprofen, suggesting that insulin-mediated androgen synthesis is less sensitive to inhibition of proinflammatory pathways.

The present study, while presenting interesting findings, has notable limitations including a small number of relatively young participants with, on average, only modestly elevated BMI. Consequently, further larger clinical trials on more diverse populations of women with PCOS are warranted. In summary, this pilot study supports the concept that hyperandrogenism may be reduced by the suppression of proinflammatory pathways.

Disclosures

The authors have nothing to disclose.

Data Availability

Some or all data sets generated during and/or analyzed during the present study are not publicly available but are available from the corresponding author on reasonable request.

Clinical Trial Information

ClinicalTrials.gov registration No. NCT04485403 (registered <date>).

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