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# Expression of steroid sulfated transporters and 3β-HSD activity in endometrium of women having polycystic ovary syndrome

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ABSTRACT

Intracrinology mechanism involves the metabolism of steroids in peripheral tissues, such as DHEA, to molecules with estrogenic or androgenic activity. Proliferation rate of endometria from Polycystic Ovary Syndrome women (PCOS) is increased, favoring hyperplasia development. Besides, in endometria from PCOS-women the synthesis of androst-5-ene-3β,17β-diol (androstenediol), an estrogenic molecule, is enhanced concomitantly to increased cellular proliferation. DHEA, the major intracrinological precursor, circulates mainly in its sulfated form and requires transporters for cell intake, that belong to the families of organic anion transporting polypeptides (OATP) and organic anion transporters (OAT). The aim of this study was to determine protein levels and activity of sulfated steroid transporters OATP2B1, OATP3A1, OATP4A1 and OAT4 in endometria from control and PCOS-women and to evaluate the activity of the enzyme 3β-HSD. Levels of transporters were done by RT-PCR (OAT4 only) and Western-blot (WB). Additionally, in primary culture cells stimulated with steroids, protein levels by WB and uptake of tritiated DHEAS, were evaluated; 3β-HSD activity was assessed using radiolabel substrate. PCOS-endometrium had higher levels of OATP2B1 and OATP4A1 than CE ($p < 0.05$); decreased OATP4A1 levels were found in androstenediol or testosterone-stimulated cells. Accordingly, the entry of DHEAS to cells was lower in cells stimulated with testosterone ($p < 0.05$); 3β-HSD-activity was similar in control and PCOS-endometria. Therefore, this study describes that steroids can modulate the expression and activity of transporters of OATPs-family in human endometria and that some transporter levels are increased in PCOS-endometria, suggesting a potential role in the pathogenesis of endometrial hyperplasia of these patients.



## 1. Introduction

Intracrinology is defined as the peripheral metabolism from a precursor of low activity, such as dehydroepiandrosterone (DHEA), to a metabolite of higher affinity for androgens or estrogens steroid receptors [1]. The steroidogenic enzymes present in peripheral tissues are essential for this metabolic process. Intracrinology is increased in certain diseases where steroids are key to favor cell proliferation, such as breast [2] and prostate [3,4] cancer. Additionally, elevated concentrations of the sulfated form of DHEA (DHEAS) are found in blood, being around 3–6 μM in premenopausal women [1].

The entry of DHEAS into cells requires transporters [5,6], which are mostly carriers belonging to the family of organic anion transporting polypeptides (OATP) and organic anion transporters (OAT). Particularly, the focus of this paper is on transporters OATP2B1, OATP3A1, OATP4A1 and OAT4 [7,8].

As reported, in several tissues including the endometrium, DHEA could be metabolized to androst-5-ene-3β,17β-diol (androstenediol), a steroid with weak estrogen activity, although associated with diseases where the estrogenic effect plays an important role in cell proliferation. In fact, it is reported that women with high serum levels of DHEA and androstenediol are at increased risk of developing breast cancer [9]. Besides, it has been described in several tumor tissues that androstenediol may stimulate cell proliferation via estrogen receptors, where intratumoral concentrations are elevated [10]. Among other tissues, the endometrium is particularly sensitive to steroid action; even more, in endometrium from women with Polycystic Ovarian Syndrome

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(PCOS) there is an increased cellular proliferation [11–13]. Interestingly, women with PCOS have high serum levels of DHEAS, although within normal range. Previous reports from our group have indicated that in PCOS endometria the metabolism of DHEA to androstenediol is increased; in addition, high tissue concentration of androstenediol was found [14,15]. However, if the androstenediol to testosterone metabolism is altered in those tissues, is still unknown. Furthermore, several anion transporters have been described in endometria from normal and PCOS women [14]; among them the protein content of the transporter OATP4A1 is increased in endometria from PCOS patients compared to normal women [14]. Endometrial protein levels of other transporters are not known, neither the possible mechanisms involved in the regulation of these proteins.

Therefore, based on the described action of androstenediol on endometrial cell proliferation in addition to the importance of the acquisition of DHEAS by these cells, the objectives of this study were to determine the protein levels, activity and mechanisms regulating sulfated steroid transporters OATP2B1, OATP3A1, OATP4A1 and OAT4 in endometria from control and PCOS women and to evaluate the activity of the enzyme 3β-HSD, which catalyzes the conversion of androstenediol to testosterone.

## 2. Experimental

Hormonal levels were assayed using commercial kits: serum testosterone, androstenedione and progesterone by radioimmunoassay (RIA) (Diagnostic System Laboratories, Webster, TX, USA); sex hormone-binding globulin (SHBG) concentration by RIA (DPC, Los Angeles, CA, USA) and 17β-estradiol by electrochemiluminescence (Roche, Basel, Switzerland).

The antibodies used for Western blot were β-actin (A5441, Sigma, USA), OATP2B1, (#ab83532 Abcam, Cambridge, UK), OATP3A1 (#ab884315 Abcam, Cambridge, UK), OATP4A1 (#HPA030669 Sigma–Aldrich Co., St Louis, MO, USA), OAT4 (#ab93168 Abcam, Cambridge, UK) and anti-rabbit secondary antibody (#074-1806, KPL, Gaithersburg MD, USA). To assay for enzyme activity was used androst-5-ene-diol 3β17β [1,2-3H(N)] (#1635, American Radiolabeled Chemicals, USA) while for the uptake assay was used dehydroepiandrosterone sulfate [1,2,6,7-3H(N)] (#NET860250UC, Perkin Elmer, USA).

### 2.1. Subjects

This investigation was approved by the University of Chile Clinical Hospital and School of Medicine, University of Chile Ethical Committees and informed written consent was obtained from all subjects, in agreement with national legislation and the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association (Declaration of Helsinki).

The endometrial specimens ($n = 16$) were classified as follows: endometria obtained during the proliferative phase of the menstrual cycle (control endometria (CE), $n = 8$) and endometria obtained from patients with PCOS (PCOSE, $n = 8$). The tissues were obtained with a pipelle suction curette from the uterine corpus from women with PCOS. The diagnosis of PCOS was made according to the Excess Androgen Society [16,17], considering clinical and/or biochemical signs of hyperandrogenism and at least one of two other criteria: oligo- and/or anovulation and polycystic ovaries. Hyperprolactinaemia (prolactin (PRL) > 35 ng $mL^{-1}$), androgen-secreting tumors (total testosterone > 2 ng $mL^{-1}$; DHEA-S > 3600 g $mL^{-1}$), Cushing's syndrome (urine cortisol concentration > 150 g 24 h and fasting plasma concentration of cortisol > 25 g $dl^{-1}$), congenital adrenal hyperplasia (17-OH progesterone > 2.5 ng $mL^{-1}$), attenuated 21-hydroxylase deficiency, diabetes and thyroid disease (thyroid stimulating hormone (TSH) > 5 U $L^{-1}$) were excluded. Endometria from patients without PCOS (CE) were obtained by hysterectomy from women with non-neoplastic pathology at hysterectomy. None of the women (control or PCOS) had received hormonal therapy, clomiphene citrate or insulin sensitizers within 3 months prior to recruitment into the study, and the endometria used in this study all showed normal morphology. The endometrial phases were evaluated by an experienced pathologist using the histological criteria of Noyes [18].

### 2.2. Tissue preparation

Endometrial tissue samples were divided into three or more pieces. One of these was embedded in paraffin and used for endometrial dating. The other two pieces were frozen in liquid nitrogen and then at −80 °C, later were used for Western blotting and enzymatic activity techniques.

### 2.3. Cell cultures

The cells for the primary cultures used for this study were obtained from endometria of a control woman without neoplastic endometrial pathology during proliferative phase. The cells were propagated in DMEM HAM F12 medium without phenol red (Sigma–Aldrich Co., Saint Louis, MO, USA) supplemented with 1.5 g/L sodium bicarbonate, 1× Insulin–Transferrin–Selenium (ITS) mixture, 500 ng/mL puromycin, 10 v/v fetal bovine serum (FBS) and cultured at 37 °C and 5% $CO_2$.

For Western blot, cells were cultured for 24 h at a ratio of 800,000 cells/plate and then treated for 48 h with 100 nM of androstenediol, testosterone or androstenediol plus testosterone in DMEM HAM F12 without serum. The cultures with no hormonal stimulation were used as the basal condition for the study. The steroids concentration used for treatment was determined in previous studies [15].

### 2.4. RNA isolation and semiquantitative RT-PCR of OAT4

Total RNA was obtained using TRIZOL (Gibco BRL, Life Technology) according to manufacturer's recommended instructions. The pieces of tissue (∼30 mg) were homogenized in 1 mL of TRIZOL, the organic phase and the aqueous phase were separated by centrifugation (16,000×g for 15 min at 4 °C) after the addition of chloroform. To precipitate the RNA, was added isopropanol to the supernatant and again centrifuged (16,000×g for 10 min at 4 °C), it was subsequently washed with 1 mL of 75% ethanol. The RNA was resuspended in diethylpyrocarbonate-water. RNA integrity was determined by gel electrophoresis in 1% agarose denaturing conditions. Complementary DNA (cDNA) was synthesized from 2 mg of total RNA treated with DNase I, using the enzyme reverse transcriptase SuperScript II (Gibco BRL, Life Technology). The reaction mix was made for a total volume of 25 μL, with 1 μg of total cDNA, 5 μL of 5× buffer, 2 mL of 25 mM $MgCl_2$, 0.5 μL of 10 mM dNTPs, 1 μL of each primer of OAT4, 0.075 μL of primers of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and 0.15 μL of GoTaq® Flexi DNA Polymerase 5 U/μL (#M8291). PCR program used consisted of 35 cycles with 94 °C denaturation for 1 min, 62 °C for 1 min for annealing and 1 min 30 s extension. Placenta cDNA was used as a positive control for the PCR, and as negative control was used water without cDNA. Reaction was performed in Termocycler PTC-100 (MJ Research Inc., Watertown, MA, USA). The nucleotide sequence for OAT4 was CCGGTGGCTGATTATTAAGGG for the sense primer and TGTTGGCTAGAATGGCGAGG for the antisense primer, generating a product of 419 base pairs. For GAPDH, the sense sequence primer was CCACCATGGAGAAGGCTGGG and antisense ATCACGCCACAGTTTCCCGG, with a

product of 287 base pairs. Then, the amplified products for all samples were separated by 1% agarose gel Red® electrophoresis and GAPDH used as a constitutive gene was also amplified. The intensity and area of the bands were analyzed with the program UN-SCAN IT gel 4.1 (Silk Scientific Corporation). The results were expressed as the ratio of OAT4 mRNA/GAPDH mRNA (AU).

### 2.5. Western blotting

The Western blot was assessed as previously reported [15,19]. In brief, fresh tissue specimens and cell samples were homogenized and lysed on ice using a cell lysis RIPA buffer (50 mM Tris, 150 mM NaCl, 0.5 v/v sodium deoxycholate, 1 v/v triton, 0.1 v/v SDS) containing a protease inhibitor cocktail (Roche, IN, USA). After centrifugation at 10,000×g for 20 min at 4 °C, protein concentrations were determined using the BCA protein assay kit (Pierce, IL, USA). Total proteins (40 μg from each sample) were denatured and fractionated using 10% one-dimensional-SDS–PAGE and transferred to nitrocellulose membrane (BioRad, CA, USA). Blots were blocked for 2 h in TBST (20 mM Tris, pH 7.6; 137 mM NaCl; 0.1 v/v Tween 20) containing 5 v/v nonfat dry milk. Subsequently, the blots were washed three times for 5 min each in TBST and then incubated overnight at 4 °C with antibodies against OATP2B1 (1:300), OATP3A1 (1:500), OATP4A1 (1:400) and OAT4 (1:800). β-Actin antibody (1:20,000) was incubated for 1 h at room temperature. The blots were washed three times for 5 min each with TBST, followed by incubation while rocking for 1 h at room temperature with anti-rabbit IgG peroxidase-linked species-specific whole antibody (1:5000) or anti-mouse IgG, peroxidase-linked species-specific F (ab′)2 fragment (1:10,000). After washing three times for 10 min each with TBST, the bound antibodies were detected with an enhanced chemiluminescence system (Western Lightning® Plus-ECL NEL103001EA, Perkin Elemer, USA). Band intensities were quantified by scanning densitometry utilizing the UN-SCAN-IT software, Automated Digitizing System, version 5.1. The results were expressed as a ratio of the housekeeping gene β-actin.

### 2.6. Enzymatic activity of 3β-HSD

The conversion of androstenediol to testosterone was performed according to the technique of Jellinck et al. [20]. Twenty to 30 mg of frozen tissue (−80 °C) were homogenized with phosphate buffer (100 mM KCl, 10 mM $KH_2PO_4$, 10 mM $Na_2HPO_4$, and 1 mM EDTA, pH 7.5), and then centrifuged at 1000g for 15 min to separate the aqueous phase (supernatant) from the organic phase. The proteins in the supernatant were quantified. A total of 100 μL of the organic phase was added to 50 μL Dulbecco PBS buffer, 50 μL of 1 mM nicotinamide adenine dinucleotide phosphate, reduced (NADPH) and 50 μL of [$^3$H]-androstenediol. The radioactivity measured from duplicate controls without samples was used for the subtraction of the background. In addition, we used two controls without NADPH to determine the effectiveness of the cofactor. The solution was incubated in a humid atmosphere of 5% $CO_2$/air at 37 °C for 3 h with agitation. The reaction was stopped with acetone/ethyl acetate (1:2.5, v/v). The mixture was vortexed and then centrifuged, the supernatant was removed and dried with nitrogen and the residue was dissolved in 0.2 ml methanol. [$^3$H]-Androstenediol was characterized sequentially by thin layer chromatography (TLC).

### 2.7. Statistical evaluation

The calculate number per group was 8 assuming $\alpha = 0.05$ and $\beta = 0.2$, a difference between means of 0.25 and standard deviation of 0.3 according to our previous studies [11,15,21]. Comparisons between groups were performed by Mann Whitney test. $p$-Values <0.05 were considered significant. Statistical tests were performed using Stata 9 and Graph Pad Prism 6.0.

## 3. Results

### 3.1. Clinical and endocrine characteristics

The concentration of steroids and clinical features of patients are shown in Table 1. As observed, patients with PCOS present high serum levels of testosterone, androstenedione, free androgen index (FAI) and DHEAS and lower levels of SHBG compared to control women, as is characteristic of the syndrome. No differences in body mass index (BMI) of women in both study groups were detected.

### 3.2. Evaluation of transcript levels and protein of transporter OAT4 in endometrium of PCOS women and controls

The transcript of OAT4 was detected in endometrium of women with PCOS and controls, through conventional RT-PCR (Fig. 1). Placenta was used as a positive control for all studied transporters and normalization was performed using the GAPDH transcript. No differences were observed when comparing the levels detected in the PCOSE group versus CE ($p = 0.24$) (Fig. 1). Likewise, protein levels analyzed by Western blotting for the two study groups showed no statistical differences ($p = 0.14$). Normalization was performed using β-actin levels in all Western blotting studies (Fig. 1).

### 3.3. Determination of protein content of transporters in PCOS women endometrial tissues and controls

Protein levels of transporters in endometrial samples were analyzed by Western blot. It was detected significantly higher protein levels of OATP2B1 in PCOSE than in CE (∼150%, $p = 0.049$) (Fig. 2). Besides, the content of OATP3A1 was increased in PCOSE compared to CE, although not statistically significant (Fig. 2). Meanwhile, OATP4A1 protein levels in PCOSE was higher than in CE (∼65%; $p = 0.007$) (Fig. 2).

### 3.4. Evaluation of the protein content of transporters in endometrial primary cultures stimulated with steroids

The protein content of OATPs in primary cultures of endometrial cells subjected to steroid treatment was evaluated. The results show that levels of neither OATP2B1 nor OATP3A1 did change with the stimuli added ($p > 0.05$) (Fig. 3). In contrast, treatment with androstenediol (∼40%) and testosterone (∼30%) exerted a decrease on OATP4A1 protein levels compared to the basal condition

**Table 1**
Clinical and hormonal characteristics of patients.

| | Control | PCOS | Ref. range |
|---|---|---|---|
| Age | 34.1 ± 1.6 | 24.8 ± 0.9[a] | |
| BMI (kg/m$^2$) | 27.6 ± 1.8 | 31.2 ± 1.5 | <25 |
| $P_4$ (ng/mL) | 0.75 ± 0.3 | 0.76 ± 0.3 | <1 |
| $E_2$ (pg/mL) | 46.6 ± 12.8 | 69.9 ± 6.3 | 30–100 |
| $A_4$ (ng/mL) | 1.6 ± 0.3 | 2.7 ± 0.4[a] | 0.8–3.1 |
| T (ng/mL) | 0.37 ± 0.06 | 0.7 ± 0.6[a] | <0.6 |
| SHBG (nmol/L) | 49.8 ± 11.1 | 25.9 ± 2.2[a] | 16–120 |
| FAI | 3.5 ± 0.6 | 10.5 ± 1.2[a] | <4.5 |
| DHEAS (ng/mL) | 1143.8 ± 184 | 2340.6 ± 424[a] | <3600 |

Control women (C) and women with Polycystic Ovarian Syndrome (PCOS), body mass index (BMI), progesterone ($P_4$), estradiol ($E_2$), androstenedione ($A_4$), testosterone (T), sex hormone binding globulin (SHBG), free androgen index (FAI), dehydroepiandrosterone sulfate (DHEAS). The values are expressed as mean ± SEM.
[a] $p < 0.05$ versus control. Mann Whitney test.

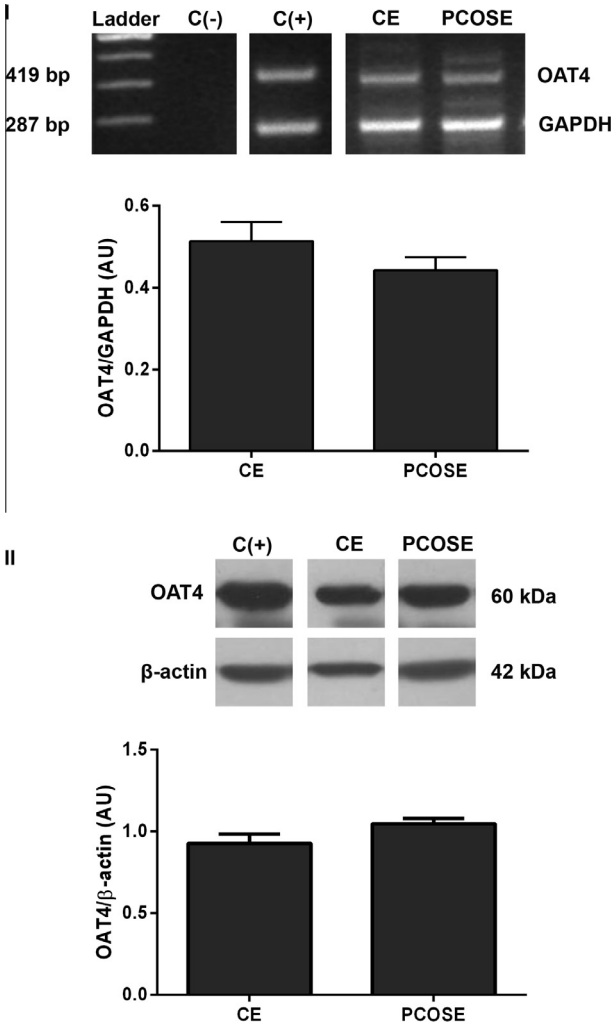


**Fig. 1.** (I) RT-PCR of OAT-4 transporter and (II) Western blot of OAT4 in endometrial tissues from control women (CE) and women with PCOS in proliferative phase (PCOSE). Placenta was used as a positive control (C (+)) and the negative control (C (−)). The semi-quantification of the transcript was normalized to GAPDH and protein levels with β-actin. $n = 8$. The results are expressed as mean ± SEM.

($p = 0.04$), whereas, the stimulus of testosterone plus androstenediol did not show differences from basal ($p = 0.42$) (Fig. 3).

The protein content of OAT4 was analyzed in primary cultures of endometrial cells treated with steroids for 48 h. The stimuli with androstenediol, androstenediol plus testosterone or testosterone alone did not cause changes in protein levels of this transporter ($p > 0.05$) (data not shown).

### *3.5. DHEAS uptake assay in primary culture cells derived from human endometria under steroids stimulation*

The uptake of DHEAS was evaluated in primary cultures of endometrial cells under steroids stimuli. It was determined that in the presence of androstenediol, estradiol or testosterone plus androstenediol the uptake activity was similar to basal ($p > 0.05$) (Fig. 4). However, testosterone alone decreased transporter activity compared to basal (~46%; $p = 0.004$). Notably, the addition of bromosulfophthalein, a non-selective inhibitor of OATPs transporters, also decreased DHEAS uptake compared to basal (~30%; $p = 0.02$); this result could indicate that the entry of DHEAS occurs principally by members of OATPs (Fig. 4).

### *3.6. Evaluation of 3β-hydroxysteroid dehydrogenase (3β-HSD) enzyme activity in endometrium of women with PCOS and controls*

The 3β-HSD enzyme activity was evaluated in all tissue samples using tritiated androstenediol. The data indicate that no significant differences were found between the two studied groups ($p = 0.29$) (Fig. 5). In the absence of cofactor, reduced levels compared to samples with cofactor were obtained (data not shown). Notably, endogenous levels of androstenediol on PCOSE [15] did not affect the results obtained in experiments of 3β-HSD activity, since a saturating concentration of radiolabeled substrate was used (Fig. 5).

## 4. Discussion

It is well known that the entry to cells of sulfated steroids is through transporters expressed in the plasma membrane of diverse types of cells. In previous unpublished work of our group using two cell lines of endometrial stroma cells, T-HESC and St-T1b, very low protein levels of the transporters OAT4, OATP2B1, OATP3A1 and OATP4A1 were found. These results were consistent with reports in other tissues, which described that these transporters are mainly expressed in epithelial and not stromal cells [8]. Therefore, in the present work primary cultures of stroma and epithelia endometrial cells were used.

Various steroids have been described as regulators of the activity of organic anion transporters. Studies with progesterone or dexamethasone indicate that these molecules are capable of increasing DHEAS entry into cells that overexpress the transporter OATP2B1 [22]; meanwhile, testosterone decreases activity of this transporter, affecting the entry of DHEAS into cells [5]. In agreement, in our study, stimulation with testosterone exerts negative effects to the entrance of DHEAS (Fig. 4). This is probably associated with the reduction in the protein levels of some transporters in the presence of this steroid observed in the present work (Fig. 3), or that testosterone could regulate the activity of certain anion transporters. Based on the fact that testosterone is not a substrate for these carriers, decreased income DHEAS could occur by allosteric regulation [5].

In this context, PCOS patients with high plasma levels of androgens and low progesterone levels, associated with ovarian failure, should present a decrease in the activity of the transporters, at least OATP2B1. Nevertheless, a previous report showed that in the endometrium of these women, OATP2B1 and OATP4A1 protein levels were elevated (Fig. 2) [14]; therefore, this increase in transporters protein molecules could potentially represent a compensatory mechanism for favoring the uptake of sulfated DHEA. Additionally, the high plasma concentration of DHEAS in PCOS women could favor its uptake (Table 1) and consequently, the intracrinological metabolism at the endometrial level. It is important to point out that the high serum levels of DHEAS in PCOS women included in the study (Table 1) are within the reference ranges and similar to the one reported in the literature [23]. Furthermore, the high levels of OATP4A1 protein detected in PCOSE by Western blot in the *ex vivo* model (Fig. 2), is consistent with the previous report of high levels of this transporter evaluated in epithelial compartment through indirect immunofluorescence technique [14].

The functional *in vitro* assays gave us valuable information about the modulator action of steroids on the activity and protein levels of sulfated steroid transporters (Figs. 3 and 4). In the present work it was determined that in endometrial cell cultures, testosterone and androstenediol decreased protein levels of the transporter OATP4A1 (Fig. 3). However, in the *ex vivo* model in the PCOS condition, where high serum levels of androgens are present,

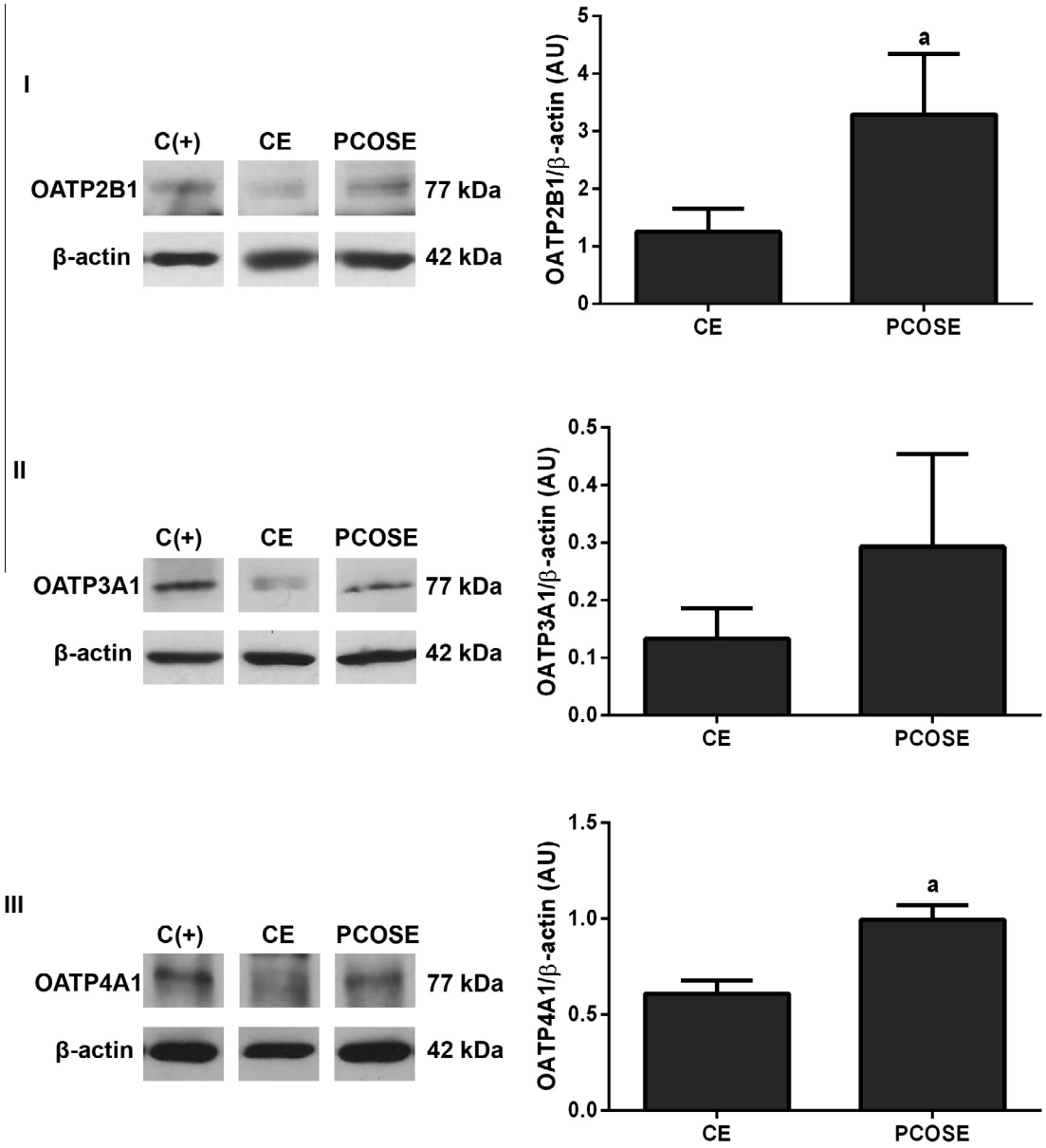


**Fig. 2.** Evaluation by Western blot technique of transporter protein levels of: I-OATP2B1, II-OATP-3A1 and III-OATP-4A1, normalized to levels of β-actin, in endometria from control women (CE) and endometria from women with PCOS (PCOSE). Placenta tissue is used as a positive control. (a) $p < 0.05$ vs. CE. $n = 8$. Results are expressed as mean ± SEM.

an increase protein levels of some of these transporters (Fig. 2) were detected. This discrepancy between the two models could reside in part given the high structural and functional complexity of the endometria, where many factors interact to assure the normality of the tissue, which are not considered in the *in vitro* model. In this regard, some molecules have been described as regulators of the expression of these transporters, particularly the subfamily OATP1B. In fact, in primary cultures of hepatocytes, interleukin 1β, interferon-γ and hepatocyte growth factor levels decrease some transporters like OATP2B1 [24–26]. This founding may be relevant since it is known that interleukin-1β is increased in adipose tissue of obese individuals [27], and a high percentage of patients with PCOS have this condition. There are studies that described that STAT5 pathway increases the levels of some members of the family OATPs [28]. Additionally, data from our group established that the endometrium of women with PCOS have high levels of VEGF [29] and as known, VEGF is able to activate the STATs pathway in the model of epithelial ovarian cancer [30,31]. Thus, it is possible that in the endometrium of women with PCOS, VEGF may increase the levels of transporters OATP2B1 and OATP4A1 by STATs pathway; however, this should be confirmed with further studies.

It is known that androstenediol originates from DHEA metabolism through the catalytic action of 17β-HSD [1], and that in the endometrium of women with PCOS this conversion is increased [14]. These reports, together with the results of the present study, suggest that androstenediol synthesis is increased in the endometrial tissue [14], although it is not converted to testosterone (Fig. 5); therefore, androstenediol is accumulated in this tissue, which is consistent with the high concentration of androstenediol detected in pathological endometrium [15]. This steroid is a strong candidate for modulating the proliferative process present in this

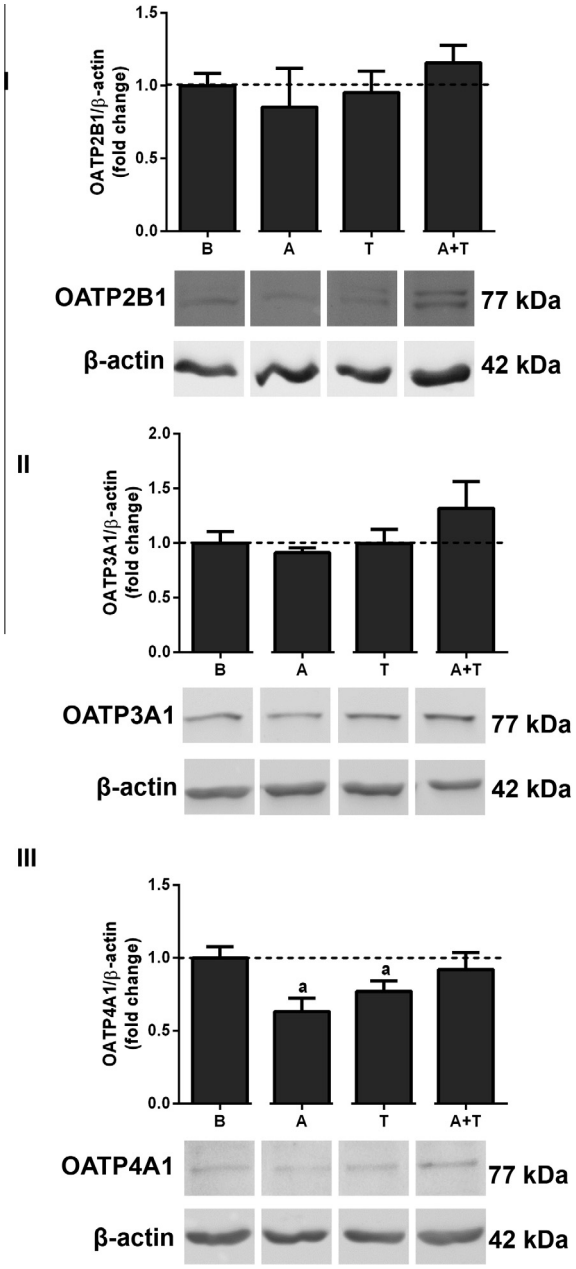


**Fig. 3.** Evaluation of transporter protein levels of: I-OATP2B1, II-OATP3A1 and III-OATP4A1 by Western blot in endometrial cells obtained from primary culture stimulated with steroids. B: Basal, A: androstenediol, T: testosterone, A + T: androstenediol plus testosterone (100 nM). The stimuli were applied for 48 h. Normalization was performed with protein levels of β-actin. (a) $p < 0.05$ vs. (B) $n = 3$ in duplicate. Results are expressed as mean ± SEM.

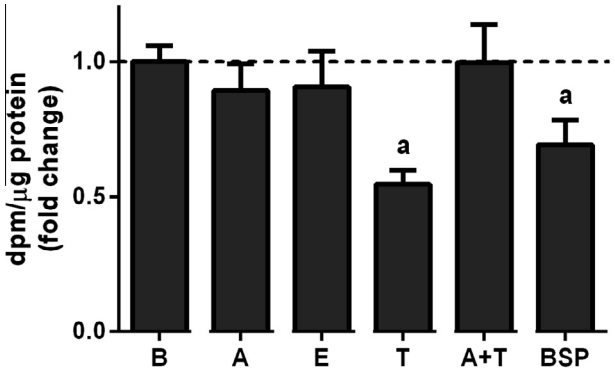


**Fig. 4.** Uptake assay of [$^3$H] DHEAS by cells obtained from a primary endometrial culture stimulated with steroids. B: Basal, A: androstenediol, E: estradiol, T: testosterone, A + T: androstenediol plus testosterone (100 nM). BSF: bromosulfophthalein. (a) $p < 0.05$ vs. (B) $n = 3$ in duplicate. Results are expressed as mean ± SEM.

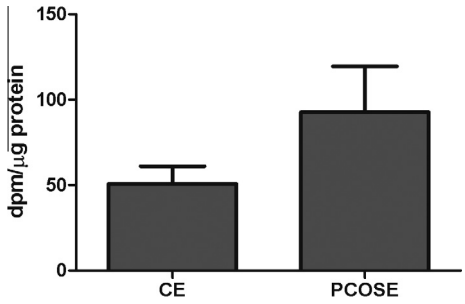


**Fig. 5.** Enzymatic activity assay of 3β-HSD in endometrial tissue from control women (CE) and women with PCOS (PCOSE). The evaluation of converting androstenediol-to-testosterone was expressed in dpm and normalized per μg of total protein. $p = 0.29$. $n = 3$ in duplicate. Results are expressed as mean ± SEM.

tissue, as this molecule has estrogenic activity and estrogens activate cycle progression and endometrial cell proliferation [15,32].

In summary, some of the sulfated steroid transporters, important for the intake of intracrine precursors, are increased in the endometrium from PCOS women, which may favor the entry of these molecules to the tissue under study, potentially these higher protein levels could favor the increase of its activity. Additionally, it was found that steroids are capable of modulating the levels and activity of some transporters. This research gives information about the first step of the intracrine pathway, being relevant since this steroidogenic pathway is increased in PCOS endometrium that could partially explain the loss of tissue homeostasis by favoring cell proliferation.

## Author contributions

F.P-P. contributed to the conception and design of the study, acquisition of data, analysis and interpretation, drafting the article and the final approval of the version to be published. C.P. contributed in the analysis of data and approved the final version. F.G. contributed in the interpretation of data, revised the article critically for important intellectual content. R.C. contributed to conception of the study, critical revision of article and approved the final draft for publication. C.R. contributed in the critical revision and final version. L.V. contributed in the conception and design of the study, analysis and interpretation of data, the critical revision and final version. M.V. conception and design, analysis and interpretation of data, revised the article critically for important intellectual content and approved the final draft for publication.

## Declaration of interest

The authors declare that there are no conflicts of interest.

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## References


[1] F. Labrie, V. Luu-The, C. Labrie, A. Bélanger, J. Simard, S.-X. Lin, G. Pelletier, Endocrine and intracrine sources of androgens in women: inhibition of breast cancer and other roles of androgens and their precursor dehydroepiandrosterone, Endocr. Rev. 24 (2003) 152–182.

[2] H. Sasano, Y. Miki, S. Nagasaki, T. Suzuki, *In situ* estrogen production and its regulation in human breast carcinoma: from endocrinology to intracrinology, Pathol. Int. 59 (2009) 777–789.

[3] J.A. Locke, E.S. Guns, A.A. Lubik, H.H. Adomat, S.C. Hendy, C.A. Wood, S.L. Ettinger, M.E. Gleave, C.C. Nelson, Androgen levels increase by intratumoral de novo steroidogenesis during progression of castration-resistant prostate cancer, Cancer Res. 68 (2008) 6407–6415.

[4] K.-H. Chang, R. Li, M. Papari-Zareei, L. Watumull, Y.D. Zhao, R.J. Auchus, N. Sharifi, Dihydrotestosterone synthesis bypasses testosterone to drive castration-resistant prostate cancer, PNAS 108 (2011) 13728–13733.

[5] M. Grube, K. Köck, S. Karner, S. Reuther, C.A. Ritter, G. Jedlitschky, H.K. Kroemer, Modification of OATP2B1-mediated transport by steroid hormones, Mol. Pharmacol. 70 (2006) 1735–1741.

[6] E.-E. Baulieu, P. Robel, Commentary: dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) as neuroactive neurosteroids, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 4089–4091.

[7] I. Tamai, J.-I. Nezu, H. Uchino, Y. Sai, A. Oku, M. Shimane, A. Tsuji, Molecular identification and characterization of novel members of the human organic anion transporter (OATP) family, Biochem. Biophys. Res. Commun. 273 (2000) 251–260.

[8] M. Roth, A. Obaidat, B. Hagenbuch, OATPs, OATs and OCTs: the organic anion and cation transporters of the SLCO and SLC22A gene superfamilies, Br. J. Pharmacol. 165 (2012) 1260–1287.

[9] J.F. Dorgan, F.Z. Stanczyk, C. Longcope, H.E. Stephenson, L. Chang, R. Miller, C. Franz, R.T. Falk, L. Kahle, Relationship of serum dehydroepiandrosterone (DHEA), DHEA sulfate, and 5-androstene-3β,17β-diol to risk of breast cancer in postmenopausal women, Cancer Epidemiol. Biomarkers Prev. 6 (1997) 177–181.

[10] N. Honma, S. Saji, M. Hirose, S.-I. Horiguchi, K. Kuroi, S.-I. Hayashi, T. Utsumi, N. Harada, Sex steroid hormones in pairs of tumor and serum from breast cancer patients and pathobiological role of androstene-3β,17β-diol, Cancer Sci. 102 (2011) 1848–1854.

[11] C. Avellaira, A. Villavicencio, K. Bacallao, F. Gabler, P. Wells, C. Romero, M. Vega, Expression of molecules associated with tissue homeostasis in secretory endometria from untreated women with polycystic ovary syndrome, Hum. Reprod. 21 (2006) 3116–3121.

[12] A. Villavicencio, K. Bacallao, F. Gabler, A. Fuentes, J. Albornoz, A. Casals, M. Vega, Deregulation of tissue homeostasis in endometria from patients with polycystic ovarian syndrome with and without endometrial hyperplasia, Gynecol. Oncol. 104 (2007) 290–295.

[13] A. Villavicencio, A. Goyeneche, C. Telleria, K. Bacallao, F. Gabler, A. Fuentes, M. Vega, Involvement of Akt, Ras and cell cycle regulators in the potential development of endometrial hyperplasia in women with polycystic ovarian syndrome, Gynecol. Oncol. 115 (2009) 102–107.

[14] F. Plaza, F. Gabler, C. Romero, D. Vantman, L. Valladares, M. Vega, The conversion of dehydroepiandrosterone into androst-5-ene-3β,17β-diol (androstenediol) is increased in endometria from untreated women with polycystic ovarian syndrome, Steroids 75 (2010) 810–817.

[15] F. Plaza-Parrochia, K. Bacallao, C. Poblete, F. Gabler, R. Carvajal, C. Romero, L. Valladares, M. Vega, The role of androst-5-ene-3β,17β-diol (androstenediol) in cell proliferation in endometrium of women with polycystic ovary syndrome, Steroids 89 (2014) 11–19.

[16] R. Azziz, E. Carmina, D. Dewailly, E. Diamanti-Kandarakis, H.F. Escobar-Morreale, W. Futterweit, O.E. Janssen, R.S. Legro, R.J. Norman, A.E. Taylor, S.F. Witchel, Position statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an androgen excess society guideline, J. Clin. Endocrinol. Metab. 91 (2006) 4237–4245.

[17] R. Azziz, E. Carmina, D. Dewailly, E. Diamanti-Kandarakis, E.F. Escobar-Morreale, W. Futterweit, O.E. Janssen, R.S. Legro, R.J. Norman, A.E. Taylor, S.J. Witchel, The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report, Fertil. Steril. 91 (2009) 456–488.

[18] R. Noyes, A. Hertig, J. Rock, Dating the endometrial biopsy, Am. J. Obstet. Gynecol. 122 (1975) 262–263.

[19] R. Carvajal, C. Rosas, K. Kohan, F. Gabler, D. Vantman, C. Romero, M. Vega, Metformin augments the levels of molecules that regulate the expression of the insulin-dependent glucose transporter GLUT4 in the endometria of hyperinsulinemic PCOS patients, Hum. Reprod. 28 (2013) 2235–2244.

[20] P.H. Jellinck, M. Kaufmann, A. Gottfried-Blackmore, B.S. McEwen, G. Jones, K. Bulloch, Selective conversion by microglia of dehydroepiandrosterone to 5-androstenediol—a steroid with inherent estrogenic properties, J. Steroid Biochem. Mol. Biol. 107 (2007) 156–162.

[21] A. Villavicencio, K. Bacallao, C. Avellaira, F. Gabler, A. Fuentes, M. Vega, Androgen and estrogen receptors and co-regulators levels in endometria from patients with polycystic ovarian syndrome with and without endometrial hyperplasia, Gynecol. Oncol. 103 (2006) 307–314.

[22] A. Koenen, K. Köck, M. Keiser, W. Siegmund, H.K. Kroemer, M. Grube, Steroid hormones specifically modify the activity of organic anion transporting polypeptides, Eur. J. Pharm. Sci. 47 (2012) 774–780.

[23] K. Brennan, A. Huang, R. Azziz, Dehydroepiandrosterone sulfate and insulin resistance in patients with polycystic ovary syndrome, Fertil. Steril. 91 (2009) 1848–1852.

[24] M. Le Vee, P. Gripon, B. Stieger, O. Fardel, Short communication down-regulation of organic anion transporter expression in human hepatocytes exposed to the proinflammatory cytokine interleukin 1, Drug Metab. Dispos. 36 (2008) 217–222.

[25] M. Le Vee, V. Lecureur, A. Moreau, B. Stieger, O. Fardel, Differential regulation of drug transporter expression by hepatocyte growth factor in primary human hepatocytes, Drug Metab. Dispos. 37 (2009) 2228–2235.

[26] M. Le Vee, E. Jouan, A. Moreau, O. Fardel, Regulation of drug transporter mRNA expression by interferon-c in primary human hepatocytes, Fundam. Clin. Pharmacol. 25 (2011) 99–103.

[27] C.J. Tack, R. Stienstra, L.A.B. Joosten, M.G. Netea, Inflammation links excess fat to insulin resistance: the role of the interleukin-1 family, Immunol. Rev. 249 (2012) 239–252.

[28] M. Wood, M. Ananthanarayanan, B. Jones, R. Wooton-Kee, T. Hoffman, F.J. Suchy, M. Vore, Hormonal regulation of hepatic organic anion transporting polypeptides, Mol. Pharmacol. 68 (2005) 218–225.

[29] K. Bacallao, F. Plaza-Parrochia, A. Cerda, F. Gabler, C. Romero, D. Vantman, M. Vega, Levels of regulatory proteins associated with cell proliferation in endometria from untreated patients having polycystic ovarian syndrome with and without endometrial hyperplasia, Reprod. Sci. (2015) 1–8.

[30] H. Chen, D. Ye, X. Xie, B. Chen, W. Lu, VEGF, VEGFRs expressions and activated STATs in ovarian epithelial carcinoma, Gynecol. Oncol. 94 (2004) 630–635.

[31] H. Yu, R. Jove, The STATs of Cancer-New Molecular Targets come of age, Nat. Rev. Cancer 4 (2004) 97–105.

[32] S.K. Munro, C.M. Farquhar, M.D. Mitchell, A.P. Ponnampalam, Epigenetic regulation of endometrium during the menstrual cycle, Mol. Hum. Reprod. 16 (2010) 297–310.