Sex hormone-binding globulin expression in the endometria of women with polycystic ovary syndrome

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Objective: To evaluate the protein and messenger RNA expression of sex hormone-binding globulin (SHBG) in endometria from women with polycystic ovary syndrome (PCOS).
Design: Case-control study.
Setting: Hospital research unit.
Patient(s): Thirty-three women with PCOS, and 17 fertile, healthy women of similar age to those with PCOS.
Intervention(s): Endometrial and blood samples were obtained from women with PCOS (PCOSEs) and from control women (CEs) during the proliferative phase of the menstrual cycle.
Main Outcome Measure(s): Expression studies for SHBG (immunohistochemistry and reverse transcription-polymerase chain reaction). Hormonal studies for determining sex steroids (T, P, and E_2) and SHBG concentration. Insulin sensitivity was assessed by composite insulin sensitivity index ($ISI_{composite}$).
Result(s): In stroma, the protein expression of SHBG was lower in PCOSEs than in CEs. Epithelial cells had a similar expression of SHBG protein in both groups. Messenger RNA of variant 548 base pairs (wild-type) tended to be lower in PCOSEs compared to CEs. When PCOSEs were classified by insulin resistance, the PCOSEs with normal insulin sensitivity showed an expression of stromal SHBG similar to that observed in CEs.
Conclusion(s): The low SHBG expression in the stromal compartment of endometria from women with PCOS with insulin resistance may contribute to generate an abnormal steroid milieu in the endometria of these women. (Fertil Steril® 2007;87:321–8. ©2007 by American Society for Reproductive Medicine.)

Key Words: Sex hormone-binding globulin, endometrium, polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders affecting women of reproductive age, and is characterized by hyperandrogenism, irregular menses, anovulation, and infertility (1–6). In addition, most women with PCOS exhibit peripheral insulin resistance, associated with compensatory hyperinsulinemia (7–12). Also, an association between PCOS and endometrial adenocarcinoma has been reported for many years (13, 14). It was postulated that a prolonged estrogen (E) action unopposed by P may be the cause of the high risk of developing endometrial hyperplasia and endometrial adenocarcinoma in women with PCOS (15–17).

Sex hormone-binding globulin (SHBG), which is a glycoprotein mainly secreted by the liver, binds sex steroids with a higher affinity for androgens than Es (18). The hepatic synthesis of SHBG is up-regulated by Es and down-regulated by androgens and insulin, and plays an important role in the control of serum steroid-free concentration, hence modifying the bioavailability of Es or androgens (19, 20). Moreover, evidence indicates that the SHBG-steroid complex may also play a direct role in intracellular steroid actions in target cells (21, 22). In women with PCOS, a low SHBG serum concentration associated with a high free androgen index was observed (23–25).

The expression of a fragment of 548 base pairs (bp) of SHBG messenger ribonucleic acid (mRNA) was observed in the female reproductive tract, including the endometrium, fallopian tube, and ovary (26–28). Moreover, it was reported that the human SHBG gene is organized into eight exons, separated by seven small introns (29). Screening of a human

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testis complementary DNA (cDNA) library revealed the presence of a clone lacking a 208-bp region as an exon 7 splicing variant of the SHBG gene (29). This clone encodes for a truncated form of 341 bp of mRNA for SHBG, which lacks a part of the steroid-binding domain. The SHBG exon-7-splicing-variant mRNA was detected in normal human uterine endometrium, ovarian endometriosis, and uterine carcinomas (30–32).

Because SHBG may represent an important regulator of endometrial-cell function, the aim of the present investigation was to evaluate the protein and mRNA expression of SHBG in endometria from women with PCOS.

MATERIALS AND METHODS

Subjects

Human endometria were obtained with a pipelle suction curette from the corpus of the uterus from 33 women with PCOS (PCOSE). The diagnosis of PCOS was made according to the Rotterdam Consensus (European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine) (33). Inclusion criteria were chronic oligomenorrhea (bleeding intervals between 35 days and 6 months) or amenorrhea (bleeding interval of 6 months), hirsutism, plasma T concentration >0.6 ng/mL or a free androgen index (FAI) >5.0, and characteristic ovarian morphology on ultrasound, based on criteria described by Adams et al. (1).

Patients with different grades of hyperinsulinemia, as evaluated by an oral glucose tolerance test (OGTT), were included. All women studied were amenorrhoeic and anovulatory, according to transvaginal ultrasound monitoring of follicular growth in one cycle, and serum P concentrations <4.0 ng/mL in midluteal phase (34). Hyperprolactinemia, androgen-secreting neoplasms, Cushing's syndrome, attenuated 21-hydroxylase deficiency, and thyroid disease were excluded by appropriate tests.

Control endometrium (CE) was obtained from 17 fertile healthy women of ages similar to those of women with PCOS (34.7 ± 6.3 (SD) years versus 31.9 ± 5.7 (SD) years during the proliferative phase of the menstrual cycle at time of bilateral tubal ligation at the San Borja-Arriarán Clinical Hospital, University of Chile, National Health Service, Santiago, Chile. Controls were selected in the proliferative phase because of the similar morphology of proliferative endometrium and PCOSE. None of the women, either controls or those with PCOS, had taken oral contraceptives or other medications for at least 6 months before starting the study.

In all women, an OGTT was performed. Briefly, after an overnight fast of 10 hours and a diet that contained 300 g/d of carbohydrate, a 75-g OGTT was performed. Serum glucose and insulin levels were measured before the glucose load and 30, 60, 90, and 120 minutes afterwards.

The insulin sensitivity index ($ISI_{composite}$) of Matsuda and DeFronzo (35) was used to evaluate insulin sensitivity from data obtained from the OGTT. This index of whole-body insulin sensitivity [10,000/square root of (fasting glucose × fasting insulin) × (mean glucose × mean insulin during OGTT)] indicates peripheral and hepatic insulin sensitivity. Women with a value of insulin sensitivity <6.0 (mean ± 2SD of control women) were considered to be insulin-resistant. Only women with normal glucose tolerance according to American Diabetes Association criteria were included in the study (36).

Moreover, in controls and women with PCOS, measurements of SHBG, T, androstenedione, and E_2 were also performed before the glucose load. The free androgen index (FAI) (FAI = T [nM/L]/SHBG [nM/L]) was calculated.

This investigation was approved by our Institutional Ethics Committee, and informed written consent was obtained from all subjects. In all tissue samples, histological dating and classification were performed, according to the criteria of Noyes et al. (37). The presence of endometrial hyperplasia was established by an experienced histopathologist, according to the criteria of Kurman et al. (38).

Biochemical Assay

Hormone determinations were assayed with the use of commercial kits: serum T, androstenedione, P, E_2, and insulin by radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA), and SHBG concentration by immunoradiometric assay (Diagnostic Products Corp.). The intra- and interassay coefficients of variation were 7.0% and 11.0% for T, 3.7% and 4.9% for androstenedione, 2.7% and 5.0% for E_2, 4.8% and 9.2% for P, 5.0% and 8.0% for insulin, and 3.8% and 7.9% for SHBG, respectively. Serum glucose was determined by the glucose oxidase method (Photometric Instrument 4010; Roche, Basel, Switzerland). The coefficient of variation of this method was <2.0%.

Tissue Preparation

Endometrial tissue samples were divided into two pieces. One piece of each sample was fixed in 4% buffered fomaldehyde for 24 hours, embedded in paraffin, and cut into 5-μm-thick sections before immunohistochemistry. The other piece was snap-frozen in liquid nitrogen and stored at −70°C for reverse transcriptase-polymerase chain reaction assay (RT-PCR).

Immunohistochemical Detection

The expression of SHBG was examined in histological paraffin wax sections. Paraffin sections were deparaffinized in xylene, and hydrated gradually through graded alcohol. Incubating the samples in 3% hydrogen peroxide for 5 minutes prevented endogenous peroxidase activity. Sections were incubated in 10 mM sodium citrate buffer (pH 6.0) at 95°C for 20 min. Nonspecific antibody binding was prevented with 4% phosphate-buffered saline-bovine serum albumin (PBS-BSA) for 1 hour. The polyclonal antibody anti-SHBG