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ORIGINAL ARTICLE

Age, body mass index, and serum level of DHEA-S can predict glucocorticoid receptor function in women with polycystic ovary syndrome

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Abstract Glucocorticoid receptor (GR) transduces the glucocorticoid (GC) signal that could lead to metabolic derangements depending on the tissue responsiveness to GC. We aimed to investigate possible causative relation of the GR functional properties in peripheral blood mononuclear cells of women with polycystic ovary syndrome (PCOS), with their clinical and biochemical characteristics. Thirty women with PCOS [mean age: 26.5 ± 5.1 years, mean body mass index (BMI) 24.5 ± 5 kg/m^2], and thirty respective controls were analyzed for the number of GR sites per cell (B_{max}), apparent equilibrium dissociation constant (K_d), and binding potency (GR potency). A strong association between B_{max} and K_d ($r = 0.70$, $P < 0.0001$), and GR potency with age ($r = 0.49$, $P = 0.009$) was observed in PCOS women. The multiple regression analyses within the PCOS group revealed that independent predictors for K_d were BMI, total cholesterol, and dehydroepiandrosterone-sulfate (DHEA-S) ($r = 0.58$, $P = 0.038$), while for GR potency ($r = 0.687$, $P = 0.013$) were age, BMI, DHEA-S, and basal cortisol concentration. The results suggest that PCOS pathophysiology may be related to alterations of a cross stalk between glucocorticoid signaling, age, and metabolic parameters. These findings should be further explored in studies on the role of GR in PCOS-related metabolic derangements.

Keywords PCOS · Glucocorticoid receptor · Glucocorticoid-receptor-binding characteristics · DHEA-S

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Introduction

Polycystic ovary syndrome (PCOS) is a common endocrinopathy of the reproductive-aged women that is characterized by hyperandrogenic features, with an estimated prevalence of 5–10% [1–3]. Besides ovaries as the main source of androgens, excess adrenal androgens and adrenocortical dysfunction were observed in women with PCOS [4–7]. The alterations in the response of the adrenal cortex were attributed to enhanced peripheral cortisol metabolism, followed by a compensatory overdrive of the hypothalamic–pituitary–adrenal (HPA) axis and a consequent increased androgen production [8, 9]. In recent years,

PCOS is considered to be a metabolic disorder with a number of obesity-related risk factors for cardiovascular disease, specifically insulin-resistance, type-2 diabetes, and proatherogenic lipid profile [10–13].

The glucocorticoid (GC) excess is linked to the metabolic derangements presented by central obesity, hyperlipidemia, and insulin-resistance [14, 15]. Explanation lies in tissue responsiveness to GCs that could be presented as higher GC sensitivity in obesity [16] or decreased sensitivity to hypercholesterolemia [17]. GCs exert their function through a cytoplasmic receptor, glucocorticoid receptor (GR), which functions as a hormone-activated transcription factor [18]. In all human tissues, after the hormone binding, GR transduces a hormonal signal by translocation into the nucleus where it can either bind to specific DNA sequences, known as glucocorticoid response elements, or interact with other transcription factors and co-factors, such as activator protein-1 (AP-1) and nuclear factor-kappaB (NF-κB) [19, 20] in a tissue-specific manner. Most of the studies on human GR are performed on peripheral blood mononuclear cells (PBMCs), as readily accessible human cells that express high levels of the receptor. It has previously been shown that transcriptome of peripheral lymphocytes reflect various pathological states, which is considered a consequence of functional integration of neuro-, endocrine-, imune- and metabolic-systems [21]. Thus, these cells can be used as a non-invasive diagnostic indicator. In PBMCs from healthy human subjects GR number per cell (B_{max}) is correlated to the equilibrium dissociation constant (K_d), implying that there is a compensatory mechanism between the GR hormone-binding capacity and affinity [22]. The GR number and its affinity for the hormone in PBMCs were assessed in a previous study on PCOS women, but the differences in these indices between PCOS and controls were not observed [23]. Recently, another parameter based on the ratio of the GR number per cell and its affinity for the hormone, called "binding potency", was introduced for the assessment of the GR functional status under the conditions of stress [24].

Therefore, in this study we aimed to analyze possible causative relation between functional properties of the GR in PBMCs and clinical characteristics of women with PCOS.

Results

Clinical characteristics of PCOS patients and controls

Clinical and biochemical characteristics of patients and control subjects are presented in Table 1. PCOS women differ from the respective controls in terms of hyperandrogenemia [testosterone ($P < 0.0001$), sex-hormone-binding globulin (SHBG; $P = 0.001$), FAI ($P < 0.0001$) and DHEA-S ($P = 0.006$)] but not in terms of insulin sensitivity nor in the indices of GR hormone-binding activity and basal cortisol concentrations.

Table 1 Clinical and biochemical characteristics of the women with PCOS and the controls

	PCOS ($n = 30$)	Controls ($n = 30$)	P
Age (years)	26.5 ± 5.1	28.1 ± 4.0	0.184
BMI (kg/m^2)	24.5 ± 5.8	23.0 ± 5.2	0.289
Waist circumference (cm)	82.1 ± 13.8	78.5 ± 14.2	0.334
Total cholesterol (mmol/l)	5.1 ± 0.9	5.0 ± 1.0	0.628
HDL-C (mmol/l)	1.3 ± 0.2	1.4 ± 0.2	0.597
LDL-C (mmol/l)	3.2 ± 0.7	3.1 ± 0.9	0.769
Triglycerides (mmol/l)	1.0 ± 0.4	0.8 ± 0.3	0.057
Glucose (mmol/l)	4.4 ± 0.3	4.4 ± 0.3	0.577
Insulin (mU/l)	14.1 ± 7.1	14.0 ± 5.5	0.953
HOMA index	2.8 ± 1.6	2.7 ± 1.1	0.705
Testosterone (nmol/l)	3.3 ± 1.3	1.6 ± 0.5	<0.0001
SHBG (nmol/l)	40.2 ± 18.9	61.1 ± 26.7	0.001
FAI	9.4 ± 4.3	3.1 ± 1.4	<0.0001
DHEA-S (μmol/l)	8.0 ± 4.3	5.4 ± 2.2	0.006
Cortisol (nmol/l)	483.7 ± 174.8	427.1 ± 145.4	0.183
B_{max} (fmol/10^6 cells)	12.2 ± 8.0	13.7 ± 7.2	0.443
K_d (nM)	34.3 ± 30.4	26.9 ± 19.6	0.294
GR potency (B_{max}/K_d)	0.6 ± 1.1	0.8 ± 0.5	0.666

In the PCOS group, BMI was positively associated with age ($r = 0.36$, $P = 0.05$) and WC ($r = 0.891$, $P < 0.0001$). Age of the women with PCOS was positively correlated with triglycerides ($r = 0.39$, $P = 0.034$), while total cholesterol concentrations were correlated with testosterone ($r = 0.43$, $P = 0.017$). Insulin concentration in the PCOS group was associated with BMI ($r = 0.63$, $P < 0.0001$) and with WC ($r = 0.46$, $P = 0.013$). SHBG was found to be in negative association with WC ($r = -0.45$, $P = 0.015$), insulin concentrations ($r = -0.544$, $P = 0.003$), and HOMA index ($r = -0.539$, $P = 0.004$).

In the women with PCOS, B_{max} was positively associated with K_d ($r = 0.70$, $P < 0.0001$), and GR potency with age ($r = 0.49$, $P = 0.009$). Besides confirmed positive correlation of B_{max} with K_d ($r = 0.75$, $P < 0.0001$) in controls, we did not observe association between GR potency and BMI, DHEAS and age in this group.

Regression analyses in PCOS patients

Binary logistic regression model gives out Nagelkerke multinomial logistic regression coefficient $R^2 = 0.508$. Overall percentage of the model that best fits in prediction

Table 2 Multiple regression analyses in PCOS women

	K_d *B*	GR potency *B*
Constant	66.194	−0.17
Age	–	0.149
BMI	1.026	−0.091
Total cholesterol	−15.212	–
DHEA-S	3.042	−0.045
Basal cortisol	–	−0.001
	$r = 0.58$	$r = 0.69$
	$P = 0.038$	$P = 0.013$

Independent variables that entered into the calculation were age, BMI, total cholesterol, triglycerides, testosterone, FAI, DHEA-S, and basal cortisol

of PCOS was 81.8%, and consisted of BMI ($P = 0.022$), insulin ($P = 0.076$), DHEA-S ($P = 0.010$), K_d ($P = 0.118$), GR potency ($P = 0.042$), and basal cortisol ($P = 0.020$).

In the multiple regression analyses, backward method, independent variables that entered into the calculation were age, BMI, total cholesterol, triglycerides, testosterone, FAI, DHEA-S, and basal cortisol. Independent predictors of K_d, as dependent parameter, were BMI, total cholesterol, and DHEA-S; while for the GR potency as dependent parameter the predictors were age, BMI, DHEA-S, and cortisol (Table 2).

Discussion

In this study, we demonstrated the existence of a positive association of the GR number per cell and its hormone-binding affinity in PBMCs from women with PCOS. It was shown that hormone-binding potency of the GR is related to the age and predicted by the age of the PCOS patients. Moreover, in the same group, BMI and DHEA-S appeared to be independent predictors of both K_d and GR potency.

The role of altered cortisol metabolism has become a matter of investigations for the explanation of the etiology of PCOS. A possible mechanism of the pathogenesis of PCOS may involve enhanced 5α-reductase activity [8, 25], dysregulation of the activity of 11β-hydroxysteroid dehydrogenase type 1 [8], and increased total adrenal steroid production [8, 26]. Recent data have suggested a positive relationship between DHEA-S metabolites in urine and total cortisol metabolites, reflecting adrenocortical hyperfunction that led to excessive secretion of both DHEA-S and total cortisol metabolites in the lean women with PCOS [25]. In concert with these observations, we did not find differences in serum cortisol concentration between the examined groups, while an increased concentration of DHEA-S was found in the PCOS group in comparison to the controls.

Previous studies have shown that age, sex, and BMI did not significantly influence the GR-binding capacity in healthy subjects [22, 27], and that GR number in PBMCs decreased with aging [28]. Gender-related differences as well as seasonal or circadian variations in the number of GR in PBMCs from healthy subjects were not significant [28]. However, the pattern of GR-binding characteristics variations was even more complex in pathological states. Thus, a normal or increased GR number with unchanged affinity for the hormone in comparison to controls, and without influence of the course of the disease was found in patients with multiple sclerosis [29] and myasthenia gravis [30]. In other conditions different GR characteristics in relation to the response to GC therapy were described [31–33]. The relationship between the age and the GR potency in patients with PCOS presented herein suggest an alteration in GR-binding characteristics that could develop from the earlier decades of life. This conclusion is certainly premature and needs further investigation on larger number of subjects.

Polymorphisms of the GR gene that increase sensitivity to GCs could lead to the change of body composition and metabolic derangements. These findings are frequently inconsistent. It was shown that life-long exposure to the allele carrying *N363S* polymorphism might be accompanied by an increased BMI [34]. GC hypersensitivity related to other frequent polymorphisms may change during lifespan. Early in life they are inducing more fat tissue and consequently increasing BMI, while later in life these effects are mostly pronounced on lean mass and could led to decrease of BMI with aging [35]. When more GR gene polymorphism variants were present in the same individual, a tendency toward higher total cholesterol and LDL-C was observed [36]. The results of this study clearly showed that the GR functional characteristics are independently predicted by the body composition. In addition, regression analyses confirmed that total cholesterol could predict the affinity of the receptor for the hormone at least within our PCOS group of women. Considering all these data together it could be assumed that in women with PCOS might exist an interplay between structural and functional properties of the GR on one side, and metabolic derangements on the other.

An association between high DHEA-S levels and higher prevalence of cardiovascular disease has been reported for females [37]. The cellular and molecular mechanisms of the biological effects of DHEA and DHEA-S are mostly unknown. A possible explanation could be that DHEA acts as an activator of GR trafficking. Namely, long-term exposure to DHEA promoted nuclear translocation of the GR due to the direct binding or indirect effects on GR [38].

Although, an existence of the DHEA or DHEA-S receptor has not been confirmed as yet, it could be supposed that other steroid receptors, like estrogen receptor, GR, or androgen receptor, could mediate adverse metabolic effects of DHEA-S in humans. This might be the case with PCOS as a condition with partial overproduction of DHEA-S, which might affect functional characteristics of GR presented in our group of examined women.

Recently a strong linear relationship between GR number and K_d has been found in a healthy human population. Taking that K_d is inversely proportional to the receptor affinity for the hormone, this relationship implied the existence of compensation between the number and the affinity of GR under normal physiological conditions [22]. In this study, the linear relationship between GR number and K_d was observed in the women with PCOS. Szuran et al. [24] introduced another novel parameter named GR potency for the assessment of the hippocampal GR functional status in the animal model. The results of these authors pointed out the sexual dimorphism in the GR-binding potency. In concert with these findings, data obtained from the regression analyses in our study revealed an existence of independent influence of DHEA-S on the GR potency in PCOS women and led us to the clue that adrenal-originated androgens might be mediators of the observed sexual dimorphism in the GR potency. The assessment of the GR mRNA level by real-time PCR, which is currently under progress in our laboratory (data not presented), would provide more information for the explanation of the regulation of HPA axis in PCOS. Further studies are needed for the elucidation of the functionality of adrenal-originated steroids and their role in PCOS.

In conclusion, a linear relationship between the GR number and the affinity for the hormone, as well as an influence of age on GR hormone-binding potency were found in PBMCs from women with PCOS. Age, BMI, and DHEA-S could predict the lymphocyte GR hormone-binding properties in the examined group of women with PCOS. These novel findings should be assessed through future studies on the role of glucocorticoid hormones receptor in PCOS and the possible consequent metabolic derangements.

Materials and methods

Subjects

Thirty women with PCOS [mean age: 26.5 ± 5.1 years, mean body mass index (BMI): 24.5 ± 5 kg/m^2] were compared with 30 age- and BMI-matched control subjects (mean age: 28.1 ± 4.0 years, mean BMI: 23.0 ± 5.2 kg/m^2) (Table 1). Patients were Caucasians and with similar cultural background. Both PCOS subjects and controls were recruited during a 12-month period from the outpatient clinic of the study-collaborating institutions. The patients were either referred to the institutions by primary care physicians, from the obstetrics and gynecology clinics, or were self-referral, for investigation of oligo- or amenorrhea, hirsutism, acne, or infertility.

The diagnosis of PCOS was made on the basis of the revised 2003 Rotterdam ESHRE/ASRM consensus criteria [39], with PCOS young women meeting two of the following three criteria: (1) oligomenorrhea or anovulation, (2) clinical and/or biochemical signs of hyperandrogenism, and (3) polycystic ovaries. Besides moderate oligo/amenorrhea, our patients had elevated serum testosterone concentration and polycystic appearance of the ovaries. Patients were investigated during the follicular phase of the menstrual cycle, while those with amenorrhea were evaluated after confirmation of low estrogen and progesterone levels. In all patients, the impaired fasting glucose (fasting venous glucose $\geq$ 6.0 mmol/l), obesity (BMI $>$ 30), pregnancy, hypothyroidism, non-classical 21-hydroxylase deficiency, hyperprolactinemia, Cushing's disease, and androgen-secreting tumors were excluded by appropriate tests. No subjects received any oral contraceptives, glucocorticoids, antiandrogens, ovulation induction agents, antidiabetic and antiobesity drugs, or other hormonal drugs for at least 3 months before the study.

The study was approved by the Institution Ethics Committee and written consent was obtained from all subjects.

Laboratory measurements

PCOS patients were examined during early follicular phase (within the first 6 days after the onset of menstruation), or randomly in the case of severe oligo- or amenorrhea. The controls were investigated in early follicular phase. In all PCOS patients and controls, blood samples were collected in the morning subsequent to overnight fast. Basal serum levels of total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), triglycerides, glucose, insulin, testosterone, sex hormone-binding globulin (SHBG), dehydroepiandrosterone-sulfate (DHEA-S), and cortisol were measured. Samples for determination of insulin, testosterone, SHBG, DHEA-S, and cortisol were stored at −20°C until the analysis.

The insulin sensitivity was calculated by the homeostasis model assessment (HOMA). HOMA was calculated using the formula [fasting insulin (mU/l) × fasting glucose (mmol/l)]/22.5 [40].

Total cholesterol (mmol/l) and triglycerides (mmol/l) were measured by standard enzymatic methods (cholesterol: cholesterol oxidase, Randox, UK; triglycerides:

glycerol-3-phosphate oxidase, Randox, UK). HDL-C (mmol/l) was measured by direct method (Randox, UK). LDL-C (mmol/l) was determined by Friedewald formula [41]. Plasma glucose (mmol/l) was determined by glucose oxidase method (Randox, UK), using the auto-analyser (Beckman, Austria). Plasma insulin (mU/l) levels were determined by radioimmunoassay [RIA INSULIN (PEG), INEP, Belgrade, Serbia]. The intra- and inter-assay CV were 2.5 and 7.7%, respectively. Serum testosterone (nmol/l) was measured by radioimmunoassay (TESTO-CT2, CIS bio international, Gif–Sur–Yvette Cedex, France). The intra- and inter-assay CV were 4.5 and 5.1%, respectively. SHBG (nmol/l) was measured by radioimmunoassay (SHBG-RIACT, CIS bio international, Gif-Sur-Yvette Cedex, France). The intra- and inter-assay CV were 3.9 and 4.7%, respectively. DHEA-S (μmol/l) was determined by radioimmunoassay (DHEAS-CT, CIS bio international, Gif–Sur–Yvette Cedex, France) with intra- and inter-assay CV 3.5 and 4%, respectively. Cortisol (nmol/l) was measured by radioimmunoassay (CORT-CT2, CIS bio international, Gif–Sur–Yvette Cedex, France) with intra- and inter-assay CV 3.8 and 4.3%, respectively. Free androgen index (FAI) was calculated from total testosterone and SHBG levels, using the formula FAI = (100 × T)/SHBG, with both testosterone and SHBG values expressed in nmol/l [42]. FAI > 8 was considered elevated.

Isolation of peripheral blood mononuclear cells

Peripheral blood was obtained by venipuncture into heparin anticoagulant. All samples were taken between 08:30 and 09:30 h. The blood was diluted with an equal volume of phosphate-buffered saline (PBS; 1.5-mM KH_2PO4, 6.5-mM Na_2HPO4, 2.7-mM KCl, 0.14-M NaCl, pH 7.2). PBMCs were prepared from whole blood by Ficoll-Paque PLUS (Amersham, UK) density gradient centrifugation. Mononuclear cells, recovered from the plasma/Ficoll interface, were extensively washed with PBS and resuspended in RPMI-1640 (Gibco, UK) medium supplemented with 10% heat-inactivated fetal calf serum (FCS) to a final density of 5×10^6 cells/ml. Cell viability was assessed by Trypan Blue exclusion and was always found to be more than 95%.

Hormone binding assay

Aliquots of lymphocyte suspension, containing 1×10^6 cells, were incubated with [^{3}H]dexamethasone (Amersham, UK; specific activity 40.0 Ci/mmol) at five different concentrations ranging from 7.5 to 120 nM in the absence and in the presence of 100-fold molar excess of unlabeled dexamethasone (Sigma) to determine total and nonspecific binding, respectively. After 120 min of incubation at 37°C, the reaction was stopped by the addition of 1-ml ice-cold PBS. After vacuum filtration (Whatman GF/C filters) and thorough rinsing with ice-cold PBS, filters were transferred into scintillation cocktail for radioactivity measurement (Rackbeta liquid scintillation counter, LKB, counting efficiency ≈ 48%). All determinations were performed in triplicate. Specific binding was calculated by subtracting the nonspecific from total binding. The number of receptor sites per cell (B_{max}, expressed in fmol/10^6 cells) and the apparent equilibrium dissociation constant (K_d, expressed in nM) were calculated by computer-assisted fitting of the saturation curves. Binding potency (GR potency) was calculated as the ratio of B_{max} and K_d [24].

Statistical analysis

All the data are given as mean ± SD. Comparisons between groups were made by using the Mann–Whitney test. Estimation of the direction and strength of the relationships between variables was made with Spearman correlation coefficient. Binary logistic regression, backward method (conditional), was performed for the assessment of the influence of each variable from the whole group of subjects for the prediction of disease (PCOS). Multiple regression analysis, backward method, was undertaken to define the relative influence of each variable on the GR indices. Level of significance was set on $P < 0.05$.

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