Serum levels of 3 α -androstanediol glucuronide in hirsute and non hirsute women

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Abstract

This study has evaluated the behaviour of 3 α -androstanediol glucuronide (3 α -diol G) in 170 women of whom 85 had polycystic ovary syndrome (PCOS), 35 had idiopathic hirsutism (IH) and 50 had regular cycles (control group). Of the women with PCOS, 45 were hirsute (PCOS-H) and 40 were non hirsute (PCOS-NH). Women in the control group were not hirsute. Hirsutism was assessed by the same physician using the Ferriman-Gallway score. The body mass index (BMI) was estimated in all of the women.

Plasma concentrations of 3 α -diol G were elevated only in hirsute patients, both with PCOS and with IH. Even in PCOS-NH, concentrations of 3 α -diol G were higher compared with controls ($P < 0.001$), but significantly lower ($P < 0.001$) than those of the PCOS-H and of the IH groups. The behaviour of 3 α -diol G was not affected by BMI.

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Introduction

Hirsutism, affecting 5–8% of the whole female population of fertile age, is an important clinical and psychological problem. Hirsutism is associated with an increased production or action of androgens. Hyperandrogenism was found in 60–80% of hirsute women, while in 10–15% circulating levels of androgens and of 17 α -hydroxyprogesterone (17OHP) were normal even after the adrenocorticotrophin (ACTH) stimulation test (idiopathic hirsutism) (1, 2). Sixty-five to eighty-five percent of women with androgen excess had polycystic ovary syndrome (PCOS) or hyperthecosis (3–5). Despite hyperandrogenism, about 30% of women with PCOS did not present with hirsutism (6).

Several studies have shown that in all hirsute women, both with PCOS and idiopathic hirsutism (IH), the activity of 5 α -reductase in the genital skin was steadily elevated (7–10).

3 α -Androstanediol glucuronide (3 α -diol G) is considered to be an excellent marker of peripheral androgen action and especially of 5 α -reductase activity (11, 12). It has been shown that the skin can form C-19 conjugates directly (13). A primary alteration in 5 α -reductase and in the androgen metabolism of the skin could be the cause of hirsutism in many cases.

The clinical use of 3 α -diol G as a marker of peripheral androgen action is, however, controversial because many authors deduce that the origin of 3 α -diol G, and therefore its plasma concentrations, reflect adrenal androgen production and hepatic metabolism (14, 15).

The aim of our study is to provide further contributions to the clinical activity of 3 α -diol G by evaluating its behaviour in a broad series of normal and hyperandrogenic women, with and without hirsutism.

Materials and methods

Subjects

Since 1994 plasma assays of 3 α -diol G have been carried out in 170 women aged 17 to 35 years. The patients were divided into the following groups: 50 normal females (control group), aged 18 to 35 years, body mass index (BMI) 21.6 ± 2.5 kg/m²; 85 women with PCOS, aged 17 to 29 years, BMI 24.3 ± 4.6 kg/m², of whom 45 were hirsute (PCOS-H) and 40 non hirsute (PCOS-NH); 35 women with IH, aged 17 to 25 years, BMI 22.5 ± 5 kg/m². All subjects were healthy and had taken no medication during the previous three months. We excluded patients with Cushing's syndrome, late onset congenital adrenal hyperplasia, prolactin secreting pituitary adenomas, and androgen secreting ovarian or adrenal neoplasia. The controls had regular ovulatory cycles (luteal phase serum progesterone levels >4 ng/ml) and no clinical evidence of hyperandrogenism. The diagnosis of PCOS was made according to the clinical (various cycle alterations, acne and hirsutism) and endocrine (chronic anovulation, elevated serum luteinizing hormone (LH), 17OHP and androgen levels, mean LH/follicle-stimulating hormone

(FSH) ratio >2) data. Insulin resistance was present in 49 (57.6%) of 85 women with PCOS, of whom 27 (60%) were PCOS-H and 22 (55%) were PCOS-NH. The insulin resistance was measured by the oral glucose tolerance test (OGTT). A normal insulinaemic response to OGTT was considered as a maximum insulinaemic level of 100 μ U/ml, as established by standard procedures in our laboratory, while a normal glycaemic response to OGTT was defined according to the criteria of the National Diabetes Data Group (16).

IH women had normal menstrual cycles and plasma levels of androgens and 17OHP, both in basal conditions and after the ACTH stimulation test. The ACTH test (administration of a single i.v. bolus of 0.25 mg synthetic ACTH (Synacten), Ciba-Geigy, Varese, Italy) was performed in women with IH when early follicular phase serum 17OHP levels were ≥ 30 nmol/l. Hirsutism was evaluated by the Ferriman-Gallway score (17) and only patients with a score ≥ 8 were considered hirsute. Women with BMI >25 kg/m² were considered overweight/obese.

Hormone assays

Plasma assays of 3 α -diol G were carried out between 0800 and 0900 h for two consecutive days in all women in the follicular phase (days 4 to 7) of a spontaneous or induced cycle. The value of 3 α -diol G in each subject expressed the average of two determinations. In 85 patients with PCOS and oligo-amenorrhoea the cycle was induced with the administration of medroxyprogesterone acetate at a dose of 10 mg daily for five consecutive days. Progesterone and C-19 derivate progestins but not medroxyprogesterone acetate, reduce *in vitro* 5 α -reductase activity in female genital skin (18). The effects of medroxyprogesterone acetate (at the highest dose) in hyperandrogenic patients with PCOS are probably related to inhibition of androgen through suppression of LH and an increase in the hepatic clearance of testosterone. Serum 3 α -diol G levels were assayed by means of the RIA method (kit from Diagnostic Systems Laboratories, Webster, TX, USA). Serum LH, FSH and sex hormone binding globulin (SHBG) levels were assayed using immuno-radiometric assay methods (kits by Radim, Rome, Italy). Other hormones were assayed using radioimmunoassay commercial kits from Diagnostic Products Corporation, Los Angeles, CA, USA (17OHP, testosterone, free testosterone) and Medgenix, Brussels, Belgium (fasting insulin). Glycaemia was assayed with the herokinase UV test (Boehringer-Mannheim test-Combination, Boehringer-Mannheim, Mannheim, Germany). Conversion factors to SI units were: 3 α -diol G, 2.136; total and free testosterone, 3.467; insulin, 7.175.

The study was carried out with the patients' consent and approved by the Ethical Committee of the University of Brescia.

Statistical methods

All data, normally distributed in each group, are expressed as means $\pm$ s.d. Analysis of variance (ANOVA) was used to evaluate differences between 3 α -diol G concentrations among the different groups. A *P* value <0.05 was considered statistically significant.

Results

The values for 3 α -diol G in all groups of patients are summarized in Table 1. Plasma levels of 3 α -diol G in all the women with PCOS (*n* = 85) were 4.8 ± 1.4 ng/ml, in women with IH (*n* = 35) they were 5.8 ± 1.1 ng/ml and in the controls (*n* = 50) they were 1.5 ± 0.5 ng/ml.

When the PCOS group was subdivided according to hirsutism, 45 (53%) of the patients were hirsute (Ferriman-Gallway score 12.2 ± 2.7) and 40 (47%) were non hirsute. The age of the PCOS-H patients was between 18 and 29 years with a BMI of 24.7 ± 4.7 kg/m², and the age of the PCOS-NH patients was between 17 and 27 years with a BMI of 23.8 ± 4.6 kg/m². The incidence of changes in the menstrual cycle (oligomenorrhoea, amenorrhoea) was not different in the two subgroups (PCOS-H: oligomenorrhoea 67%, secondary amenorrhoea 33%; PCOS-NH: oligomenorrhoea 67.5%, secondary amenorrhoea 32.5%). Obesity was also equally distributed in the two subgroups: 18 (40%) PCOS-H patients (BMI: 29.5 ± 3.2 kg/m²) and 14 (35%) PCOS-NH patients (BMI: 27.5 ± 1 kg/m²).

The endocrine profile for PCOS-H did not differ significantly from that for PCOS-NH in total testosterone (1.2 ± 0.4 ng/ml vs 1.1 ± 0.3 ng/ml), free testosterone (3.6 ± 0.9 pg/ml vs 3.3 ± 0.8 pg/ml) and SHBG (22.0 ± 8.0 nmol/l vs 24.2 ± 7.5 nmol/l), but it differed significantly in fasting insulin (11.7 ± 3.5 μ U/ml vs 9.3 ± 3 μ U/ml) (*P* <0.01) and 3 α -diol G levels (6.1 ± 1.7 ng/ml vs 3.5 ± 1.0 ng/ml) (*P* <0.001). In the 35 women with IH (Ferriman-Gallway score 12 ± 2.3), obesity was present in 5 cases (14.3%) (BMI: 28.4 ± 5 kg/m²) and hormone values were similar to the control group for total testosterone (0.5 ± 0.1 ng/ml vs 0.4 ± 0.2 ng/ml), free testosterone

Table 1 Serum 3 α -diol G concentrations in PCOS-H, PCOS-NH, IH and control groups. Values are means $\pm$ s.d.

	<i>n</i>	3 α -diol G (ng/ml)
PCOS-H	45	$6.1 \pm 1.7^*$
PCOS-NH	40	$3.5 \pm 1.0^{*\dagger}$
IH	35	$5.8 \pm 1.1^*$
Control	50	$1.5 \pm 0.5^{*\dagger}$

* *P* <0.001 PCOS-H and IH vs PCOS-NH and control.

$\dagger$ *P* <0.001 PCOS-NH vs control.

Table 2 Serum 3 α -diol G concentrations in the PCOS-H, PCOS-NH and IH patients subdivided by weight into obese and normal weight patients. Values are means $\pm$ S.D.

	PCOS-H		PCOS-NH		IH	
	Obese	Normal	Obese	Normal	Obese	Normal
Number of patients	18	27	14	26	5	30
BMI (kg/m ²)	29 $\pm$ 3.2	21 $\pm$ 2.8	27.5 $\pm$ 1.0	21 $\pm$ 1.4	28.4 $\pm$ 5.0	21.2 $\pm$ 1.8
3 α -diol G (ng/ml)	5.9 $\pm$ 1.5	6 $\pm$ 1.7	3.5 $\pm$ 0.9	3.4 $\pm$ 1.2	5.6 $\pm$ 0.8	5.8 $\pm$ 1.2

(2 $\pm$ 0.6 pg/ml vs 1.8 $\pm$ 0.6 pg/ml), SHBG (45.3 $\pm$ 6.1 nmol/l vs 47 $\pm$ 9.6 nmol/l) and fasting insulin (5 $\pm$ 1.9 μ U/ml vs 5.1 $\pm$ 1.1 μ U/ml). The levels of 3 α -diol G in the women with IH were significantly greater than in the control group (5.8 $\pm$ 1.1 ng/ml vs 1.5 $\pm$ 0.5 ng/ml) (P < 0.001).

Statistical analysis (Table 1) showed that serum 3 α -diol G levels in hirsute women, both with PCOS and IH, were significantly higher (P < 0.001) than those of PCOS-NH and controls. The serum 3 α -diol G levels in PCOS-H were not different from those in IH while there were significant differences (P < 0.001) between PCOS-NH and controls.

Table 2 shows the values for 3 α -diol G in PCOS-H, PCOS-NH and IH groups when each is divided into obese or normal weight patients according to their BMIs. In each group of patients, no significant variations in plasma levels of 3 α -diol G were observed between obese and normal weight women.

Discussion

Our results have shown that higher levels of 3 α -diol G are found only in hirsute patients, and are independent of androgen levels (hyperandrogenic subjects n = 45, normal androgenic subjects n = 35) and of BMI. Even values for 3 α -diol G in PCOS-NH were higher compared with the controls although significantly lower than those in hirsute women.

Therefore, in women, different plasma concentrations of 3 α -diol G can be established, ranging from normal (1.5 $\pm$ 0.5 ng/ml) to intermediate (3.5 $\pm$ 1.0 ng/ml) or very elevated values ($\geq$ 5.8 $\pm$ 1.1 ng/ml). The different quantities of 3 α -diol G probably express various degrees of 5 α -reductase activity (or possibly different types of 5 α -reductase) and different concentrations of dihydrotestosterone (DHT) in the pilosebaceous unit.

It is known that the activity of 5 α -reductase can be stimulated not only by circulating levels of androgens, but also by genetic factors (19), insulin and the insulin-like growth factor-I/insulin-like growth factor-binding protein (IGF-I/IGFBP) system (20).

Many studies have proved that in PCOS and in IH there is an increased 5 α -reductase activity in the genital skin and that plasma levels of 3 α -diol G are correlated to the activity of 5 α -reductase and to the production of DHT (21–23).

In the literature and in our series, 30% (20) and 47% of women with PCOS did not have hirsutism. Therefore, the concentrations of 3 α -diol G (3.5 $\pm$ 1.0 ng/ml) found in the PCOS-NH group expressed an increase in 5 α -reductase and in DHT which was not sufficient to induce hirsutism. In PCOS, the increase in androgens and in the insulin/IGF-I/IGFBP system probably contributes to the increase in 5 α -reductase activity and the production of DHT, but is not the main cause of hirsutism.

Hirsutism only appears when plasma levels of 3 α -diol G are very elevated, giving evidence of maximum 5 α -reductase activity and of an elevated formation of DHT in the hair follicle.

The levels of 3 α -diol G in IH and in PCOS-H, compared with PCOS-NH, suggests the importance of genetic factors in the regulation of 5 α -reductase and the key role of peripheral events in the determination of hirsutism.

The correlation between 3 α -diol G and hirsutism, and the values of 3 α -diol G in controls and in PCOS-NH, suggests a cutaneous origin of 3 α -diol G. Our results, obtained from a broad series, confirm the clinical usefulness of 3 α -diol G as a marker of the peripheral metabolism of androgens. The 3 α -diol G dosage can be employed to monitor various therapies for hirsutism.

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