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Urinary steroids in men with male-pattern alopecia

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Abstract

Enzyme hydrolysis, solid phase extraction, methoxym-silyl derivatization and capillary gas chromatographic analysis were used to examine the changes in urinary steroid metabolites in men with androgenic alopecia.

A total of 23 men with androgenic alopecia and 7 age-matched control healthy men collected 24-h urine.

Significantly increased values were found in the metabolites of testosterone (T): androsterone (A) ($p < 0.02$), and etiocholanolone (E) ($p < 0.05$) in patients with androgenic alopecia, compared to the control values. Elevated levels of 16-hydroxy-dehydroepiandrosterone (16-OHD) ($p < 0.03$) and cortisol (F) ($P < 0.05$) were found, but the levels of cortisol metabolites were unchanged. Calculating the ratio of total 5α/5β metabolites provided information on the activity of 5α-reductase. The ratio of total 5α/5β metabolites was increased in the patients showing the increased 5α-redutase activity. The elevated 16-OHD level could be indicative of patients who had mild hyperadrenal activity.

Keywords: Capillary gas chromatography; Solid phase extraction; Enzyme hydrolysis; Methoxym-silyl derivatization; Male-pattern alopecia; Steroid metabolites

1. Introduction

Alopecia can be due to different causes ranging from systematic diseases to scalp autoimmunity, the most common form is male-pattern baldness or *alopecia androge-*

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netica, i.e. androgenic alopecia, occurring after the age of puberty, in male and female, too [1,2]. The loss of hair is started at the temporal region and the two-side of the top of the head. Our investigation was focused on male-pattern alopecia (MPA) in men.

It is generally accepted that male-pattern alopecia is a direct consequence of both androgenic stimulation and genetic predisposition [3]. In the males, multifactorial genetic mechanisms have been suggested to be relevant for the development of MPA [4]. Both elevated and normal androgen levels have been reported [5–8].

At a normal level of androgen, the sensibility of end-organs, the hair bulbs or the level of sex hormone binding globulin (SHBG) can be changed. Decreased level of SHBG in serum seems a common finding in both sexes [9].

The possible origin of elevated androgen levels is the extraglandular production (skin-produced metabolites) and glandular production (testis, adrenal gland) [7].

The manifestation of the genetically determinated MPA in the male can be due to increased local extraglandular androgen metabolism in balding area. The 5α-reductase activity, the enzyme converting testosterone (T) to dihydrotestosterone (DHT), has been reported to be increased in scalp hair follicles. It has been proposed that elevated activity of the 5α-reductase may lead to higher intrafollicular DHT levels, which, in turn, inhibit hair follicle adenylate cyclase activity [10]. Increased androgen receptor binding has been found in sebaceous gland of the balding scalp [11].

The elevated androstenedione level and the unchanged levels of major androgens suggest mainly peripheral increase of androgen metabolism in males with MPA, this is supported by elevated estradiol (E2) level in the male stems from peripheral conversion of androgens to estrogens [7].

Schmidt et al. [7] concluded that suprarenal stimulation and hypophyseal feedback mechanism seem to be involved in MPA. Suprarenal hyperandrogenemia was documented by elevated levels of dehydroepiandrosterone sulfate (DHEAS) in a small group of young affected males by Pitts [5]. Phillipou and Kirk [3] found no correlation between degree of MPA and circulating serum androgen levels, maybe their large day-to-day fluctuation is a possible cause for the lack of correlation, and there were no differences in androgen levels in serum between normal and premature balding males reported by Legro et al. [8]. Burton et al. [2] found no correlation between plasma T and degree of MPA. However, the saliva T concentration was significantly elevated compared to control group according to Cipriani et al.'s findings [9].

Besides the numerous observations in serum or plasma, little information is available on steroid hormone levels in urine of men with MPA. As opposed to the serum steroid level, urinary steroid metabolites give a comprehensive overview on disorders of steroid metabolism.

Urinary steroid measurement established a positive correlation between the degree of MPA and levels of dehydroepiendrosterone (DHEA) or tetrahydrocortisone (THE) and also indicated that large numbers of patients had mild hyperadrenal activity [3].

In this study, urinary steroid excretion has been compared in men with MPA and in age-matched control men.

2. Materials and methods

2.1. Subjects

The 24-h urine samples were collected from 23 men with MPA (aged 25.1 ± 7.7 years) and from 7 control men (aged 32.4 ± 9.9 years). The men with MPA were outpatients of the Department of Dermatology, Pécs University Medical School. The control men were age-matched healthy laboratory workers.

2.2. Extraction

Twenty milliliters of urine was extracted on Sep-pak C_{18} cartridges (Waters Assoc., Milford, MA, USA). The C_{18} cartridges were primed with 5 ml of methanol and 5 ml of water, after passing through the urine, the cartridge was washed with 5 ml water and steroids were eluted with 5 ml of methanol.

2.3. Hydrolysis

The methanol extract was dried under N_2 stream, dissolved in 4 ml of 0.1 M acetate buffer (pH 4.6) to which 50 μl of β-glucuronidase/aryl sulfatase enzyme (from Helix pomatia) (Merck, Darmstadt, Germany) was added. Hydrolysis proceeded for 48 h at 37 °C. Steroids were extracted as described in Section 2.2. After evaporation under N_2 stream, the extract was dissolved in 2 ml of methanol.

2.4. Derivatization

The 100 μl extract was placed in a 10 ml glass tube with a plastic screw cap and Teflon liner, then 5 μg of the three internal standards was added (5α-androstane-3β,17β-diol (IS), stigmasterol (SS) and cholesterol butyrate (KB). The samples were dried and four drops of 2% methoxyamine hydrochloride (Sigma, St. Luis, MO, USA) in pyridine were added. After 2 h of incubation at 60 °C, pyridine was blown off and seven drops of trimethylsilylimidazole (Pierce Chemical, Rockford, IL) were added. The derivatization proceeded overnight at 100 °C yielding methoxyme-trimethylsilyl esters (MO-TMS).

2.5. Extraction on Lipidex 5000 column

Lipidex 5000 columns were prepared in Pasteur pipettes as follows: Lipidex 5000 was washed two times with 5 ml of methanol, then with 5 ml of ethanol, and two times with 5 ml cyclohexane. One hundred millimeter-column was prepared in Pasteur pipettes and was washed two times with 0.5 ml cyclohexane and once with 0.5 ml cyclohexane/pirydine/hexamethyldisilazane 98:1:1 (CPH). The extracted steroids were dissolved in 1 ml of cyclohexane and were passed through on the column, then were extracted four times with 1 ml of CPH. The extracts were concentrated to 100 μl under N_2 stream [12–14].

2.6. Chromatography

Gas chromatographic analysis was carried out on Hewlett-Packard 5890 Series II gas chromatograph equipped with flame ionisation detector, on an Ultra-1 column (25 m × 0.2 mm × 0.33 μm). The temperature program was as follows: initial temperature 50 °C was held for 2 min, then increased to 180 °C at 30 °C/min. After a 4-min isotherm period, the temperature was increased to 300 °C by 2.1 °C/min, and maintained for 8 min. The splitless injection mode was employed [14].

2.7. Measured components

The following steroid metabolites were measured: Androsterone (A); Etiocholanolone (E); Androstanediol (5α-AD); Dehydroepi-androsterone (DHEA); Androstenediol (Δ5-AD); 11-keto-androsterone (11-OA); 11-hydroxy-androsterone (11-OHA); 11-hydroxy-etiocholanolone (11-OHE); 16-hydroxy-DHEA (16-OHD); Pregnanediol (PD); Pregnanetriol (PT); Pregnenediol (Δ5-PD); Androstenetriol (Δ5-AT); Tetrahydro-11-deoxycortisol (THS); 11-keto-pregnaneteriol (11O-PT); Pregnenetriol (Δ5-PT); Tetrahydrocortisone (THE); Tetrahydro-11-dehydrocorticosterone (THA); Tetrahydro-corticosterone (THB); Allo-tetrahydrocorticosterone (aTHB); Tetrahydrocortisol (THF); Allo-tetrahydrocortisol

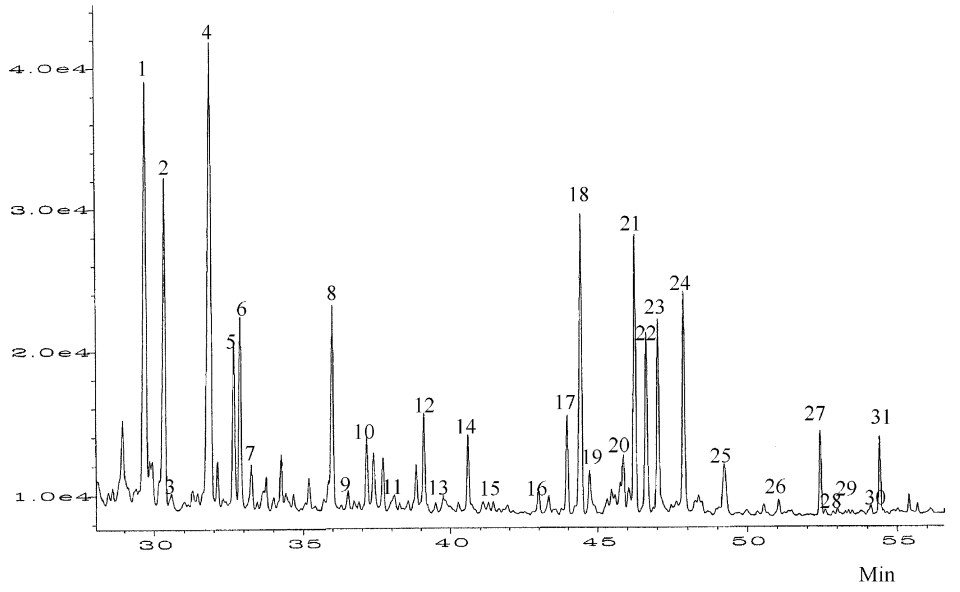

Fig. 1. Gas chromatographic separation of urinary steroids in a 44-year-old man with male-pattern alopecia. Peak identification: (1) A, (2) E, (3) 5α-AD, (4) DHEA, (5) Δ5-AD, (6) IS, (7) 11-OA, (8) 11-OHA, (9) 11-OHE, (10) 16-OHD, (11) PD, (12) PT, (13) Δ5-PD, (14) Δ5-AT, (15) THS, (16) 11-OPT, (17) Δ5-PT, (18) THE, (19) THA, (20) THB, (21) aTHB, (22) THF, (23) aTHF, (24) α-CL, (25) β-CL, (26) α-C, (27) SS, (28) F, (29) 6β-OHF, (30) 20α-OHF, (31) KB.

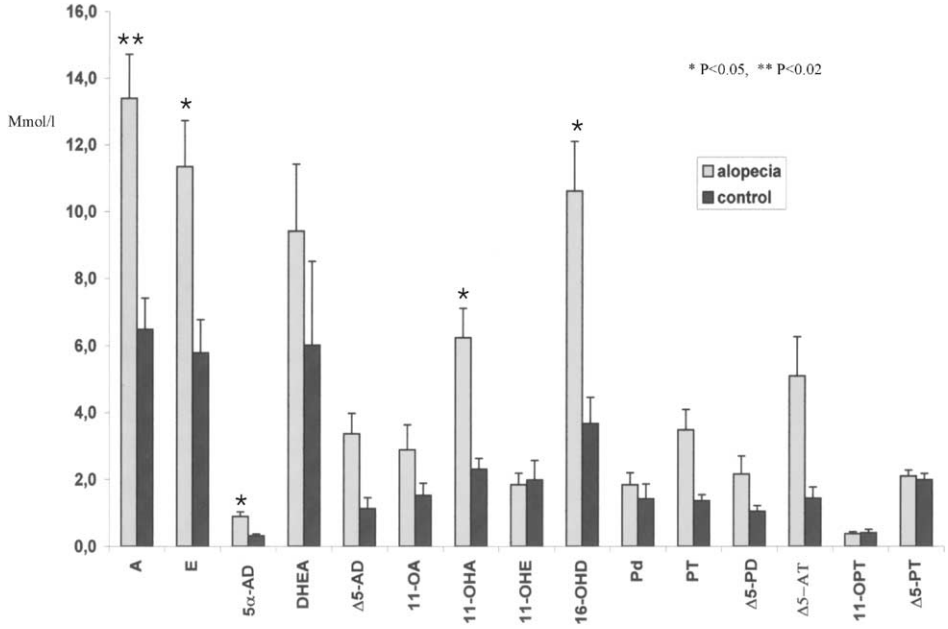

Fig. 2. Mean values ± S.E.M. of urinary excretion of androgen and pregnane metabolites in patients and control men. * $P < 0.05$; * * $P < 0.02$.

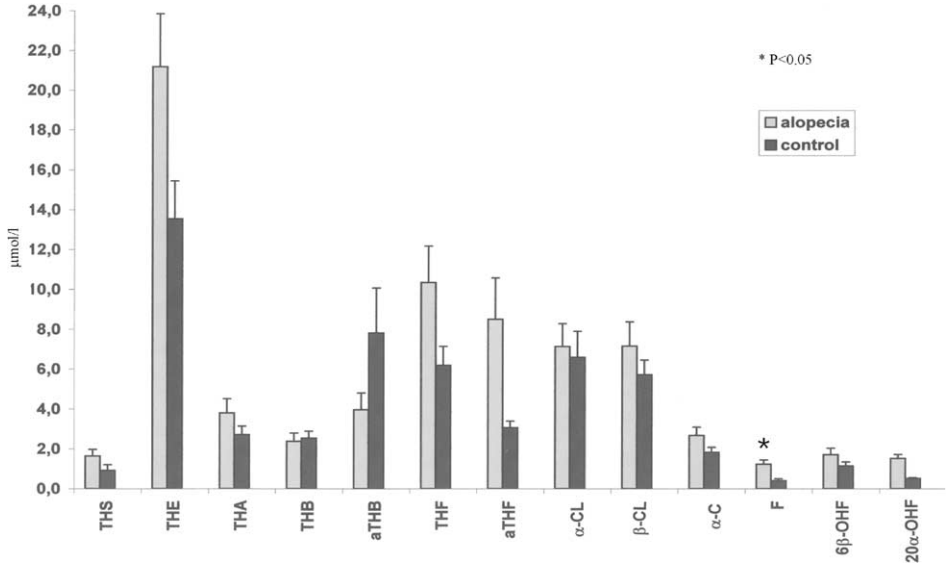

Fig. 3. Mean values ± S.E.M. of urinary excretion of cortisol metabolites in patients and control men. * $P < 0.05$.

(aTHF); α-cortolone (α-CL); ß-cortolone (ßCL); α-cortol (α-C); Cortisol (F); 6ß-hydroxycortisol (6ß-OHF); 20α-hydroxycortisol (20α-OHF).

As an index of relative activity of 5α-reductase enzyme, the ratio of total 5α reduced (A + 11β-OHA + aTHF) and total 5β reduced (E + 11β-OHE + THE + THF) metabolites were calculated.

2.8. Evaluation

Student's *t*-test was applied to an evaluation of the values.

3. Results

Separation of urinary steroid metabolites in a 44-year-old man with male-pattern alopecia is shown in Fig. 1.

The mean values of 23 men with male-pattern alopecia and 7 control healthy men and standard error of mean (S.E.M.) of daily urinary excretion of androgen and progesterone metabolites are displayed in Fig. 2. Significantly increased levels of A, E, 5α-AD, 11-OHA and 16-OHD were measured in men with MPA ($p < 0.05$).

The mean values and standard error of mean (S.E.M.) of daily urinary excretion of cortisol and corticosterone metabolites are shown in Fig. 3. Only the cortisol level was significantly elevated ($P < 0.05$) in men with MPA, compared to the control level.

The calculated ratio of total 5α/5β metabolites was also significantly ($P < 0.05$) increased.

4. Discussion

In contrast to the androgen alopecia in women, less information on the MPA in men is available.

On examining the function of the end-organ, higher formation of 5α-reduced metabolites (DHT, androstenedione) and 17-ketosteroid metabolites was observed at all sites of the scalp of bald men as compared to hair obtained from corresponding sites of women and nonbalding men [10] leading to the conclusion that androgen-dependent hair growth must be determined at a level other than T metabolism.

Schmidt et al. [7] examined the role of the hormonal parameters in androgenetic hair loss in the male and concluded that the suprarenal stimulation and the hypophyseal feedback mechanisms maybe involved in MPA. They found significant elevated androstendione levels and F level in serum of hair loss patients, compared to controls.

Increased androgen binding capacity in sebaceous glands in scalp of male-pattern baldness was observed by Sawaya et al. [11].

Only few data were available on urinary metabolites in MPA. Phillipou and Kirk [3] established positive correlation between the degree of MPA and levels of urinary DHEA or THE and attributed it to a hyperadrenal activity in a large number of patients (about 40%).

Our examination on urine steroids gives comprehensive information on the steroid metabolism. The increased A and E levels are consistent with the data obtained by Schmidt et al. [7], who found elevated serum androstenedione level, as these two compounds are metabolites of androstenedione. The elevation of androstanedione seems to point to the importance of mainly peripheral androgen metabolism [7]. Schmidt et al. [7] also found elevated serum F level, similarly, we found significantly ($P < 0.05$) higher F levels in urine in patients with MPA. These data together with the elevated 16-OHD level in our experiments are indicative of the hyperadrenal function in patients with MPA.

Steroid 5α-reductase is a membrane-bound enzyme that is responsible for the conversion of T, the major circulating androgen in adult males, into DHT. One of the known two isozymes, type I, occurs predominantly in the skin and liver, while the other, type II, is found predominantly in the prostate, genital skin fibroblast and liver [15]. In our experiments, the calculated total 5α/5β ratio was increased in men with MPA, showing the increased function of the 5α-reductase enzymes. Our results point out the role of the liver enzyme in the MPA.

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