Androstanediolglucuronide: A parameter for peripheral androgen activity before and during therapy with cyproterone acetate

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Abstract. Serum 5α-androstane-3α,17β-diolglucuronide (3α-AdiolG) levels were measured and the degree of hirsutism was scored in female outpatients complaining of excessive hair growth before and during treatment with cyproterone acetate. In a group of 16 patients with idiopathic hirsutism and in a group of 9 patients with either polycystic ovary syndrome and hirsutism or 21-hydroxylase deficiency and hirsutism, the serum 3α-AdiolG levels were significantly increased (p<0.01) as compared with the serum 3α-AdiolG level in a control group of 13 apparently healthy women: 3α-AdiolG levels, median (range), being 5.3 (2.3-7.8) nmol/l, 8.5 (4.1-10.4) nmol/l, and 2.9 (1.5-5.2) nmol/l, respectively. In contrast to a previous report, no correlation was found between the serum 3α-AdiolG levels and the Quetelet Index (N=18, R=0.42, p>0.05), indicating an apparent ineffectiveness of the excessive androgen turnover in fat tissue. The use of the anti-androgen drug cyproterone acetate alone or in combination with ethinylestradiol in reverse sequential therapy did lower the 3α-AdiolG levels significantly (p<0.01) together with a significant decrease (p<0.01) in hirsutism score. From the results of this study we therefore conclude that 3α-AdiolG can be used as a parameter for peripheral androgen action before and during treatment with anti-androgens.

Serum 5α-androstane-3α,17β-diolglucuronide (3α-AdiolG) is a metabolite of the potent androgen dihydrotestosterone (DHT; 1-4). DHT is formed in target tissue by 5α-reduction of testosterone and androstenedione (Δ4) and is metabolized in situ to androstanediol (3α-Adiol) and 3α-AdiolG or glucuronidated to DHT-glucuronide (DHTG) which is also reduced to 3α-AdiolG (3-5). The conversion of DHT to 3α-AdiolG is an essentially irreversible process, which makes formation of the glucuronide a biologically effective disposal mechanism (3). 3α-AdiolG is most likely formed almost exclusively in peripheral tissues at sites of androgen action (e.g. hair follicles), since no gradient could be demonstrated across the splanchnic bed (liver) in subjects undergoing catheterization procedures (1). Also in vitro 3α-AdiolG was found to be synthesized from testosterone in rat sexual tissue but not in rat liver (6).

Serum DHT levels do not seem to reflect the events taking place in the skin (7,8). In fact, plasma values are in the normal or near normal range in a majority of hirsute patients, a finding not unlike that for other androgens like testosterone, Δ4, and dehydroepiandrosteronesulphate (DHEAS) in hirsute women (7-9). In contrast, several investigators demonstrated elevated levels of 3α-AdiolG in women with hirsutism. Hence, 3α-AdiolG would be a reliable parameter for increased formation of androgens in target tissues (1,2,8,10). Cyproterone acetate, used in the treatment of hirsutism, has been proven to induce considerable clinical improvement of hirsutism in concordance with a reduction of serum androgen concentrations and 5α-reductase activity, measured in vitro in skin homogenates (11-14). However, to our knowledge, no report on the effect of cyproterone acetate on serum 3α-AdiolG levels in hirsute women has yet been published.

This study was undertaken to investigate serum 3α-AdiolG determination as a parameter for pe-
ripheral androgen activity before and during treatment with cyproterone acetate.

Subjects and Methods

Subjects
All patients referred to St. Joseph Hospital in the past 4 years for evaluation and treatment of unexplained hirsutism were studied. The degree of hirsutism was scored in accordance with a modification of the Ferriman & Gallwey scoring index (15; upper lip, chin, chest, breast, midline abdomen, thigh, lower leg, lower and upper arms) and the serum 3α-AdiolG concentration was determined. The women were diagnosed as having idiopathic hirsutism, based on ultrasonography of the ovaries and determination of LH, FSH and androgen concentrations. Casual LH and FSH levels were within the reference range for all women. The median (range) testosterone and androstenedione concentrations were 2.5 (1.0-5.7) nmol/l and 5.6 (3.0-10.5) nmol/l, respectively. All women of this group had a regular menstrual cycle. In the group of women with either a polycystic ovary syndrome or 21-hydroxylation deficiency and hirsutism the testosterone and androstenedione concentrations were 5.0 (2.8-12.0) nmol/l and 6.3 (5-14.3) nmol/l, respectively. In both groups thyroid disorders were excluded by normal TSH and/or free T₄ levels. Women were treated with cyproterone acetate (50-100 mg/day) or with a combination of cyproterone acetate, 50-100 mg/day and ethinylestradiol, 50 µg/day (reverse sequential therapy; 18). During treatment, the degree of hirsutism was again scored and the serum 3α-AdiolG concentration monitored. Our control group consisted of 13 women who stated that they had neither taken any medication nor suffered from excessive hair growth. The results were evaluated retrospectively.

Reagents
All reagents were of analytical grade and were used without prior purification. Lipidex-5000 was purchased from Packard Instrument Company (Zürich, Switzerland). Before use, the lipidex was dried and dissolved in a petroleum ether (64-69°C) and chloroform mixture (80:20 v/v). Unlabelled 3α-Adiol and 3α-AdiolG were obtained from Makor (Jerusalem, Israel) and Sigma (St. Louis, MO), respectively, and tritiated 3α-Adiol with a specific activity of 37.0 GBq/l was obtained from Amersham International (Buckinghamshire, England).

β-glucuronidase was obtained from Boehringer Mannheim (Mannheim, FRG). Saccel anti-rabbit was purchased from IDS Ltd (Boldon, England). Adiol antiserum (rabbit) was obtained from Biogenesis (Bornemouth, England).

Determination of serum 3α-AdiolG
Serum levels of 3α-AdiolG were measured basically as previously described (10,16,17). Characteristics of the assay: Unconjugated steroids were removed by extraction of 1 ml serum with 1 ml diethylether-ethylacetate (90:10 v/v). Hydrolysis was performed by incubation of the serum for 40 h at 45°C with 20 000 U β-glucuronidase. Separation of 3α-Adiol from other steroids was performed on a 50 cm x 3 mm diameter column packed with lipidx. The elution was carried out with petroleum ether (64-69°C)-chloroform (80:20 v/v). Fractions containing 3α-Adiol (located with tritiated 3α-Adiol in a separate experiment) were collected in glass tubes and dried at ambient temperature under a stream of nitrogen. The residue was redissolved in phosphate buffered saline, pH=7.4, containing 0.1% gelatine (PG-buffer). Polypropylene disposable tubes, containing tritiated steroid (24 000 dpm/100 µl PG-buffer), standard (0.14-3.9 pmol/100 µl PG-buffer) or sample (100 µl) and antibody (100 µl) were incubated for 24 h at room temperature. Bound steroid was separated from unbound steroid by

![Graph](image-url)

Fig. 1. Androstaneadiol G (3α-AdiolG) levels in a control group (N=13), patients with idiopathic hirsutism (IH; N=16), and patients with polycystic ovary syndrome and hirsutism or 21-hydroxylase deficiency and hirsutism (PCO/21HD; N=9). ▽=PCO, △=21HD. Median (range) 2.9 (1.5-5.2) nmol/l, 5.3 (2.3-7.8) nmol/l and 8.5 (4.1-10.4) nmol/l, respectively.
rapid addition of 100 µl of Saccel. Equilibrium was obtained within 30 min. After addition of 1 ml aquadest, centrifugation and removal of the supernatant, 100 µl 6 mol/l urea was added to dissociate the antibody-antigen complex, the mixture was vortexed and the samples were counted. Unlabelled 3α-AdiolG was used to determine recovery. Mean recovery (±SD) was 100% (±6).

Results

Serum 3α-AdiolG levels in the control group, in 16 women with idiopathic hirsutism and in 9 women with either polycystic ovary syndrome or 21-hydroxylase deficiency and hirsutism (PCO/21HD) are shown in Fig. 1. The median (range) serum 3α-AdiolG concentrations were 2.9 (1.5-5.2) nmol/l, 5.3 (2.3-7.8) nmol/l, and 8.5 (4.1-10.4) nmol/l, respectively. The median 3α-AdiolG concentration in the group with idiopathic hirsutism and in the group with PCO/21HD were significantly increased as compared with the median 3α-AdiolG concentration in the control group (Wilcoxon rank sum test; p<0.01), although there was a considerable overlap between the groups. In contrast to a previous report (19), no significant correlation was found between the serum 3α-AdiolG level and the Quetelet Index (QI; Fig. 2). The hirsutism score before and during treatment with cyproterone acetate in 16 patients (discriminated in duration of therapy) is shown Fig. 3. The median hirsutism score before treatment was 14.5 (range 7-27) and 9 during treatment (range 2-20). The decrease in hirsutism score was statistically significant (signed rank test; p<0.01). The serum 3α-AdiolG concentration before and during therapy in 15 patients is shown in Fig. 4. The median 3α-AdiolG concentration before treatment was 6.5 nmol/l (range 3.5-10.9) and 3.1 nmol/l during treatment (range 1.3-6.4). The decrease in serum 3α-AdiolG level was also statistically significant (signed rank test; p<0.01).

3α-AdiolG

Hirsutism score

![Fig. 2. Scattergram of Quetelet Index (QI) and androstanediol G (3α-AdiolG) level. N=18, Spearman's R=0.42, p>0.05.](image)

![Fig. 3. Hirsutism score according to Ferriman & Gallwey (15) before and during treatment with cyproterone acetate. ○: treatment≥ 1 year; □: treatment <1 year. Before treatment: median (range) 14.5 (7-27). During treatment: median (range) 9 (2-20).](image)
Exaggerated androgen turnover in peripheral tissues has been reported as an important cause of hirsutism in women. DHT is formed from testosterone through 5α-reductase activity in sexual skin and further metabolized to 3α-AdiolG (1-5). Previous investigators report consistently elevated serum 3α-AdiolG levels in hirsute women (2,10). Therefore 3α-AdiolG may serve as a marker of peripheral androgen action, although the exclusive extrasplanchnic production of 3α-AdiolG is sometimes questioned (19,20). In our study the median serum 3α-AdiolG level in the group of normal women is comparable with that found by others (10,19,21) using comparable techniques.

In our group with idiopathic hirsutism the median 3α-AdiolG level was significantly increased, although there was a considerable overlap between the individual 3α-AdiolG levels in the group of idiopathic hirsute patients and the control group. When the results in the PCO/21HD group were compared with those of the control group, a less pronounced overlap was seen, making 3α-AdiolG a useful marker for this group. The wide range of individual 3α-AdiolG levels in the 3 groups could be explained by the fact that the individual hirsutism score varied considerably in the 2 groups of hirsutes and that the group of normal women was not scored. Furthermore, there was no statistically significant correlation between the hirsutism score and the 3α-AdiolG level, an observation also reported by others (19). Recent reports (19,22) note another biochemical marker for hirsutism, namely serum androsteroneglucuronide, which is also a DHT metabolite and circulates at much higher concentrations in serum than 3α-AdiolG. However, although androsteroneglucuronide is elevated in hirsute women, the serum levels again do not correlate with the severity of hirsutism. The lack of correlation of the serum 3α-AdiolG levels and the Quetelet (N=18, Spearman’s R=0.43, p>0.05), confirms that adipose mass, though being a site of increased extragonadal production of androgens and their metabolites, immediately clears the excessive amounts of androgens, so that the hair follicle is not stimulated to grow (23). In a recent report (19) Scanlon et al. demonstrated a slight but significant correlation (N=22, linear regression R=0.48, p<0.05) between the serum 3α-AdiolG concentration and Body Mass Index, suggesting that weight by itself may be an important factor in determining serum 3α-AdiolG levels.

Cyproterone acetate, initially developed as a progestational compound, is a well-known anti-androgen and is used both in idiopathic hirsutism and in hirsutism owing to PCO/21HD with excellent results (11,12,13,24), which are confirmed by the results in this study. Our results are in concordance with the proposed mechanism by which cyproterone acetate acts peripherally in hirsute patients: it competes with testosterone for receptor binding sites (25,26) and it decreases 5α-reductase activity in the androgen dependent skin (13). In addition to the peripheral antiandrogen effect, cyproterone acetate has a strong antigonadotropic effect and depresses LH secretion and, through this, testosterone production in the ovary (26). Furthermore, direct activity on the adrenal is suggested (14), but also denied because the plasma testosterone and

Discussion

Exaggerated androgen turnover in peripheral tissues has been reported as an important cause of hirsutism in women. DHT is formed from testosterone through 5α-reductase activity in sexual skin and further metabolized to 3α-AdiolG (1-5). Previous investigators report consistently elevated serum 3α-AdiolG levels in hirsute women (2,10). Therefore 3α-AdiolG may serve as a marker of peripheral androgen action, although the exclusive extrasplanchnic production of 3α-AdiolG is sometimes questioned (19,20). In our study the median serum 3α-AdiolG level in the group of normal women is comparable with that found by others (10,19,21) using comparable techniques.

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Δ4 levels remained above normal in 21HD patients treated with cyproterone acetate, and ACTH-induced andrenocortical response was unchanged when using cyproterone acetate (24,27).

In summary, cyproterone acetate lowers the androgen delivery to and the androgen turnover in the target cells. We demonstrated that cyproterone acetate or cyproterone acetate-ethynylestradiol in reverse sequential therapy lowers the serum 3α-AdiolG concentration considerably in hirsute patients owing to PCO/21HD and in idiopathic hirsutism, together with a marked decrease in hirsutism score.

Therefore, we conclude that 3α-AdiolG reflects peripheral androgen turnover and may serve as a parameter for the peripheral androgen activity before and during treatment with anti-androgens.

Acknowledgments

We would like to thank Jan Valkenburg for his technical assistance.

References


Received August 27th, 1990.
Accepted December 6th, 1990.

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