3α,17β-Androstanediol Glucuronide in Plasma

A MARKER OF ANDROGEN ACTION IN IDIOPATHIC HIRSUTISM

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ABSTRACT Biologically active androgens and peripheral androgen metabolites in plasma were measured in 25 women with idiopathic hirsutism (IH). Plasma testosterone was not significantly elevated. Free testosterone however was increased although the elevation was not impressive (10.9±6.6 SD vs. 3.3±1.5 ng/dl) and one-fourth of the cases had normal unbound testosterone. Dihydrotestosterone (DHT) values were elevated (23.5±14 vs. 12.5±3.59) but again over half of the values were within the normal range. In our series of mild to moderate cases, 3α-diol was not at all discriminatory. However, plasma 3α-diol glucuronide was markedly increased (604±376 vs. 40±10 ng/dl), and elevated in all but one mild case. Previous studies document that DHT is the important androgen in skin and formation of DHT and 3α-diol is markedly increased in vitro in IH. Since 3α-diol glucuronide is derived largely from extrasplanchnic events, β-glucuronidase is present in skin, and androgen stimulates formation of the enzyme in extrasplanchnic tissue, we conclude that 3α-diol glucuronide is a marker of peripheral androgen action and markedly elevated in IH.

INTRODUCTION

Idiopathic hirsutism (IH)[1] is a common clinical entity that has puzzled endocrinologists despite advancement in hormone assay technology and knowledge of androgen physiology. It would seem that there is a subtle increase in androgen production, particularly when testosterone is measured by special techniques (1, 2). In addition, androgen prehormones such as androstenedione, dehydroepiandrosterone, and Δ5-androstenediol, are also increased in many patients (3, 4).

A second aspect of this disorder involves the hair follicle or pilosebaceous unit. This dermal tissue is not only the target for circulating androgens; there is evidence that the target is not passive but is capable of processing precursor steroids into potent androgens (5–7).

Testosterone has a dual action. As an anabolic hormone in muscle, it acts directly without being further metabolized (8). In sexual tissue such as skin or prostate, however, testosterone is converted via 5α-reduction to dihydrotestosterone (DHT) considered to be the active hormone (6, 9, 10). DHT is then 3α-reduced to 5α-androstane 3α,17β-diol (3α-diol) and then further metabolized by steroid conjugation or other pathways (11, 12). In a series of studies we have demonstrated that DHT and 3α-diol in plasma originates from peripheral conversion of secreted precursors (testosterone and androstenedione) (13, 14). Our most recent studies suggest that DHT, 3α-diol and 3α-diol glucuronide in plasma are derived in large part from extrasplanchnic metabolism and that >90% of the glucuronide originates from a pool separate from blood 3α-diol (15, 16).

These findings encouraged us to study IH as a disorder where the production of peripheral androgen metabolites may better reflect the target tissue (skin) disorder.

METHODS

Subjects. 25 women meeting the general criteria for IH were studied. These patients had moderate hirsutism as classified by Casey (17). Menstrual patterns were minimally disturbed, serum luteinizing hormone was normal, and ovaries were not enlarged on pelvic examination. The patients were identified by the above criteria as they appeared in clinic and were not selected on the basis of androgen levels.

Assays. Plasma was obtained from subjects in the morning by three withdrawals over a 1-h period and equal aliquots were pooled to reduce the effect of short-term episodic changes. Total testosterone was measured by specific radioimmunoassay (RIA) (18).

Androstanediol (3α-diol) and DHT were measured by highly specific RIA previously described by us (19, 20). Purification of the extracted steroid was by a celite column where 3α-diol and DHT are clearly separated from testosterone and other C-19 ketosteroids.

Received for publication 12 January 1982 and in revised form 16 February 1982.

[1] *Abbreviations used in this paper:* DHT, dihydrotestosterone; IH, idiopathic hirsutism; RIA, radioimmunoassay.

3α-diol glucuronide was also measured as previously described (16). Additional characteristics of the assay include: precision 9%, nonspecific blank 6±3 pg, and recovery 86%. In one key experiment, three normal and three IH samples were purified in the usual manner. Two-thirds of the sample obtained was then further isolated in the Bush A paper chromatography system for 18 h. Values obtained were minimally altered and within the precision of the assay. This paper system completely separates 3α-diol from testosterone, DHT, and the C-19, 17 ketosteroids.

Unbound testosterone was measured by the sex hormone binding globulin precipitation method of Stumpf (21). This technique quantitates at 37°C the nonsex-hormone binding globulin-bound testosterone in serum including free and albumin-bound steroids. In vivo studies indicate that albumin-bound steroids are extracted by splanchnic organs similar to free steroids (22).

RESULTS

Unconjugated androgens. Total testosterone values in the 25 patients were not significantly increased over normal values from nonhirsute, normal follicular phase ovulating women of matched age (22–34 yr), $n = 16$. Total testosterone was only 50±22 (SD) ng/dl (range, 20–95) compared with normal values of 35±10 ng. The mean IH values were higher but only one-third of the patients values were outside 2 SD of the mean of normal women (Table I).

Free testosterone was therefore of greater interest. The values were more discriminating and they were 10.9±6.6 ng/dl (range, 3–25) vs. normal values of 3.3±1.5 ng ($P < 0.001$). However, even this important parameter does not distinguish all patients from normal. One-fourth of the cases had normal unbound testosterone values (<6.3 ng). The correlation between total and free testosterone was higher than between any other parameter in the study ($r = 0.79$, $P < 0.01$). 5α:androstane 3α,17β-diol (3α-diol) was not at all discriminatory in our series of IH patients. Values were 4.3±3.5 ng/dl (range, 1–13). Again, there was a high degree of overlap between IH and normal plasma levels (3±3 ng/dl). DHT values were elevated (23.5±14 vs. 12.5±3.5, $P < 0.05$) however, the elevation was not impressive and over half of the values were within the normal range.

Conjugated androgens. In contrast, plasma 3α-diol glucuronide was markedly increased in nearly all patients (24/25). The values obtained were 604±376 ng/

TABLE I

Clinical Degree of Hirsutism, Plasma Androgens, and 3_α-diol Glucuronide in Patients with Idiopathic Hirsutism

No.	Hirsutism (1–4+)	Testosterone	"Free" testosterone	3_α-diol	3_α-diol G	DHT
				ng/dl		
1	+3	41	11.0	1.3	651	48
2	+3	83	5.8	9.5	576	41
3	+2	34	9.0	1.3	1,327	50
4	+2	95	23.0	3.4	425	—
5	+2	40	7.0	5.1	325	—
6	+1	32	3.5	1.5	167	—
7	+2	35	5.0	1.1	73	10
8	+2	40	8.0	4.0	73	30
9	+2	90	15.0	1.7	66	20
10	+1	38	10.0	1.4	33	—
11	+2	60	15.0	3.0	891	—
12	+1	35	7.0	2.0	553	11
13	+3	40	8.0	13.6	1,000	—
14	+1	20	5.0	8.0	418	10
15	+3	48	8.9	5.2	823	21
16	+3	30	4.2	2.3	381	—
17	+2	76	18.0	2.0	485	32
18	+3	32	8.6	8.0	721	20
19	+3	82	25.0	10.7	1,010	—
20	+2	37	7.7	—	1,000	—
21	+1	24	3.0	4.0	774	21
22	+3	48	9.6	—	1,300	25
23	+2	82	22.0	3.2	497	—
24	+3	61	23.0	1.4	551	5
25	+2	50	10.0	5.0	1,030	9

100 ml (range, 33–1327) vs. 40±10, $P < 0.001$ (Fig. 1 and Table I).

DISCUSSION

The possibility that local production of DHT is important has been a key concept in elucidating the pathogenesis of benign prostatic hyperplasia in men where DHT appears to be the growth hormone for this tissue. In this disorder, alterations in peripheral androgens occur in the tissue, whereas only hints of these events can be observed in the circulation (23). Idiopathic hirsutism may be another example where major changes in androgen metabolism and content takes place in the target tissue. This has been suggested by many studies showing that plasma testosterone in these patients are minimally increased.

Our study confirms many previous reports that total testosterone measurements in plasma is a poor reflection of the clinical state. We found that two-thirds of the patients had normal values. This is not to say that testosterone production is normal since careful studies by isotopic techniques indicate that many of these patients have increased blood production rates as a result of reduced sex hormone binding globulin levels and increased metabolic clearance (1). Our study also indicates that testosterone production is altered since plasma unbound testosterone was significantly increased in the IH patients. However, these values are only minimally increased and one-fourth of the patients had normal levels of unbound testosterone.

Surprisingly, the values in our series of patients for 3α-diol were not increased as others have noted mild but significant increases (24). Perhaps it is because we excluded all possible cases with polycystic ovaries. DHT, however, was increased in our group of patients.

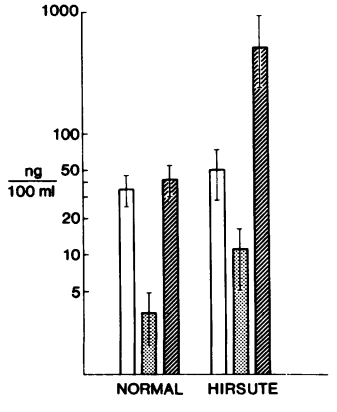

FIGURE 1 Plasma total and "free" testosterone together with androstanediol glucuronide (3α-diol G) in normal women and in idiopathic hirsutism. The height of the bars represent the mean±1 SD. □, testosterone; ▨, free testosterone; ▨, 3α-diol-G.

We (20) and others have noted variable increases in IH. Nevertheless, as for testosterone and 3α-diol, some patients had normal values.

Since DHT and 3α-diol can be derived from non-testosterone precursors such as androstanedione,Δ5-androstenediol, and even dehydroisoandrosterone, there are additional possibilities that these potential prehormones may be the source of some potent peripheral androgens. Meikle reported that adrenal stimulation increased plasma 3α-diol without altering plasma testosterone (or DHT), yet minimal gradients of 3α-diol across the adrenal could be detected (24).

Clearly, the most striking finding of our study is that plasma 3α-diol glucuronide was markedly increased in 24/25 patients in IH. The degree of elevation was very impressive and was as much as 15-fold over normal, yielding values within the male range. In vitro the formation of 5α-reduced steroid from skin in IH has been noted to approach male values (25).

Mauvais-Jarvis considered that steroid 5α- and 3α-reduction of C-19 17β-ol steroids were characteristic of peripheral androgen target tissues. This group observed that much more labeled testosterone injected was converted into 5α- and 5β-androstanediols in urine after puberty in boys. However, in the complete androgen resistance syndrome (testicular feminization), despite normal testosterone levels, the formation of 3α-diol glucuronide was reduced. Testosterone was a better precursor of urinary 3α-diol when given through skin than when given directly into the circulation (26).

These important observations were extended by us when we demonstrated that 3α-diol and its glucuronide is primarily formed by extrasplanchnic tissue. Formation of steroid glucuronides has been generally considered to take place in the liver (and gut). However, the enzyme has been located in tissues including kidney, spleen, thyroid, and sexual accessory tissue (27, 28). β-glucuronidase has also been identified in lysosomes present in epidermal cells and the enzyme was identified in another study in sweat and sebaceous glands (29). Chung and Coffee (30) recently found that rat liver exclusively forms testosterone glucuronide, while sexual tissue synthesizes 3α-diol 17β-glucuronide. Formation of the latter was inhibited by cyproterone. Well-known is the stimulating effect of androgen on renal β-glucuronidase formation. However, this induction of β-glucuronidase occurs only in extrahepatic tissue (31) and involves other androgen target tissue such as the preputial gland of the rat, which is considered to be related to sebaceous glands of the skin (32).

Therefore, we would propose that 3α-diol glucuronide is formed in peripheral tissue, especially the skin in excess in idiopathic hirsutism. Our previous work first directly suggested that plasma 3α-diol and 3α-diol

glucuronide arises from different extrasplanchnic pools in men. The present study reaches a similar conclusion in women and supports the measurement of 3α-diol glucuronide in idiopathic hirsutism as a marker of peripheral androgen action.

ACKNOWLEDGMENT

This work was supported by grant AM13710 from the National Institutes of Health.

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