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# THE EFFECT OF SPIRONOLACTONE ON GENITAL SKIN 5α-REDUCTASE ACTIVITY

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**Summary**—The effect of spironolactone (S) on genital skin 5α-reductase activity (5α-RA) of hirsute women (HW) *in vivo* as well as in normal genital skin *in vitro* was evaluated. Thirteen HW (Ferriman–Gallwey score of $23.3 \pm 2.8$) received S 100 mg twice a day for a month. Twenty-three non-hirsute women were selected as controls for the assessment of genital skin 5α-RA. S was added to incubations of genital skin from 9 additional controls *in vitro* in concentrations from $1.2 \times 10^{-8}$ to $10^{-5}$ M. HW had significantly higher conversion ratios (CR) of T to DHT compared to controls ($P < 0.05$). Post treatment values for the CR T to DHT were significantly lower than prior to S ($17.5 \pm 1.7$ and $8.05 \pm 1.2\%$, $P < 0.05$) and the mass of DHT produced also decreased by $37 \pm 9\%$ ($P < 0.05$). The CR T to 3α-diol decreased by $30 \pm 9\%$ ($P < 0.05$). In 11 of 13 women, a significant reduction of 5α-RA was demonstrated while in 2 patients the activity remained unchanged. The maximum *in vitro* inhibitory effect of S on the CR T to DHT occurred with a concentration of $1.2 \times 10^{-5}$ M ($P < 0.01$).

In conclusion, S has a direct inhibitory effect on 5α-RA. The beneficial effect of S treatment in HW may be related, in part, to this inhibition of 5α-RA.

## INTRODUCTION

Spironolactone (S) has been used effectively for the treatment of hirsutism [1–7]. The antiandrogenic activity of S is related, in part, to its effect of decreasing glandular steroid production via inhibition of cytochrome P-450 [8] as well as in increasing the clearance of testosterone (T) [1]. We have hypothesized, however, that the major antiandrogenic effect of S is its effect on peripheral androgen metabolism [5]. S has been shown to inhibit dihydrotestosterone (DHT) receptor binding [9, 10] and to be more potent than cyproterone acetate (CPA) or cimetidine in this inhibition [11].

Previous studies by Mauvais-Jarvis [13] and ourselves [14, 15] have demonstrated elevated skin 5α-reductase activity (5α-RA) in hirsute patients. Genital skin 5α-RA has been shown to be an important determinant of hirsutism and to correlate well with both the presence as well as the severity of hirsutism [14]. Recently, Mowszowicz and coworkers [12] have demonstrated that CPA, which has been used successfully in the treatment of hirsutism, inhibits 5α-RA.

This study was designed to assess the effect of S on genital skin 5α-RA of hirsute women (HW) as well as to determine its effect on 5α-RA of normal genital skin *in vitro*.

## EXPERIMENTAL

### *Subjects*

Thirteen HW, aged 21–42 participated in this study after giving informed consent. Hirsutism was scored according to the method of Ferriman and Gallwey (FG) [16] and was $21 \pm 1$ (SEM) in these patients. Five patients were diagnosed as having idiopathic hirsutism and 8 women met our criteria for polycystic ovary syndrome (PCO) as previously defined [15]. All patients received S (Aldactone®, G. D. Searle, Chicago, IL) 100 mg twice a day.

Twenty-three nonhirsute premenopausal women, aged 28–45, undergoing elective pelvic surgery for benign disease were selected as controls for the evaluation of 5α-RA. In these patients, the FG score was $5.3 \pm 0.1$ (SEM).

### *Evaluation of 5α-RA*

Genital skin biopsies were obtained from the posterior margin of the left labia majora in all patients and controls and was obtained before and 1 month after S in the hirsute group. The biopsy sites were identical in location from hirsute patients biopsied before and after S. Procedures for tissue collection, storage and incubation have been described previously [14]. In brief, the specimens were placed in a chilled container with RPMI 1640 medium (Gibco Labs, Grand Island, New York; Biological Co.,

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Santa Clara, CA) and immediately stored at −20°C for not longer than 2 weeks. Preceding the incubation, skin was thawed, freed of subcutaneous tissue and hair follicles and minced at 4°C. Four nmol of [$^{14}$C]T was added to freshly made RPMI-1640 media and skin minces were incubated in a Dubbnoff incubator for 2 h in an environment of 95% $O_2$ and 5% $CO_2$ at 37°C. Following the incubation, steroids were extracted with ether and separated by celite and paper chromatography using tritiated steroids for recovery estimates. After the separation process, radioactivity in specific eluates, dihydrotestosterone (DHT) and 5α-androstane, 3α-17-β diol (3α-diol) were quantified. In our previous publication [14], we have demonstrated that cofactors are not required, that a 2 h incubation is optimum for the recovery of DHT together with 3α-diol, and that the 5α-RA of skin is stable at −20°C for 2 weeks. The conversion ratios of DHT and 3α-diol from [$^{14}$C]T in 2 h incubations, based upon 200 mg of skin, were used as indices of skin 5α-RA and 5α-3α-ketoreductase activity. Aliquots of the chromatographic eluates for DHT were also submitted to RIA in order to estimate the mass of DHT produced (pmol/mg/2 h).

*Effects of S* in vitro

The effect of S *in vitro* was investigated in normal human genital skin obtained from 9 additional controls. Spironolactone (SC9420-Lot L-4184, G. D. Searle, Chicago, IL) was added to the incubations in concentrations of $1.2 \times 10^{-8}$ to $1.2 \times 10^{-5}$ M). Skin minces from each subject were divided into 20–30 mg portions for separate incubations with [$^{14}$C]T without S, as well as with varying concentrations of S ($10^{-5}$ to $10^{-8}$ M). In initial experiments of skin minces incubated without [$^{14}$C]T, DHT production (by RIA) was negligible. Similarly, S did not interfere with the RIA for DHT.

## RESULTS

HW had significantly higher conversion ratios of T to DHT compared to controls ($P < 0.001$). One month after the administration of S (200 mg per day), 5α-RA was significantly reduced (Fig. 1). However, after treatment of HW with S, the conversion of T to DHT was still significantly higher than values in controls ($P < 0.05$). The mass of DHT produced in HW was significantly greater ($4.42 \pm 0.96$ pmol/mg/2 h) than in controls ($0.48 \pm 0.08$; $P < 0.01$). The post-treatment values for DHT were significantly lower than those obtained prior to S ($2.10 \pm 0.36$ pmol/mg 2 h; $P < 0.02$) [Data not depicted].

In 11 of the 13 women, a significant reduction of 5α-RA was demonstrated while, in 2 patients, the conversion of T to DHT remained unchanged (Fig. 2). In 6 of the patients, 5α-RA was normalized to levels found in controls (Fig. 2).

The conversion of T to 3α-diol was significantly higher in patients before S treatment ($9.26 \pm 0.78$%) compared to controls ($2.96 \pm 0.32$%; $P < 0.02$). Spironolactone effectivly reduced this enzymatic activity significantly ($6.16 \pm 0.32$% after S; $P < 0.05$; Fig. 3).

The percent change of 5α-RA in HW after S was $46 \pm 7$% for the conversion of T to DHT was $37 \pm 9$% for DHT produced and $30 \pm 9$% for the conversion

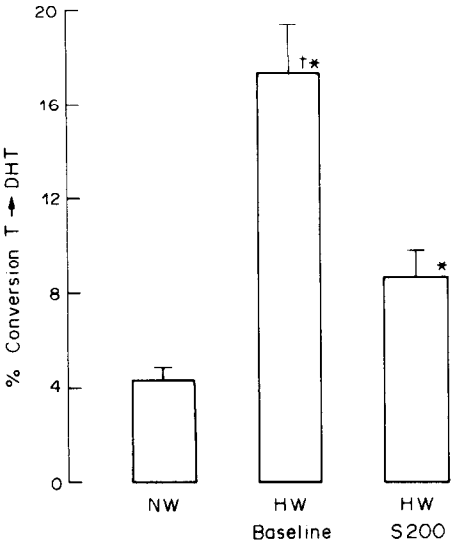


Fig. 1. The conversion of T to DHT in normal women (NW), hirsute women (HW) and in HW after 200 mg of spironolactone. *Compared to NW (HW Baseline, $P < 0.001$, HW S 200, $P < 0.05$). †HW Baseline vs HW S 200 ($P < 0.001$).

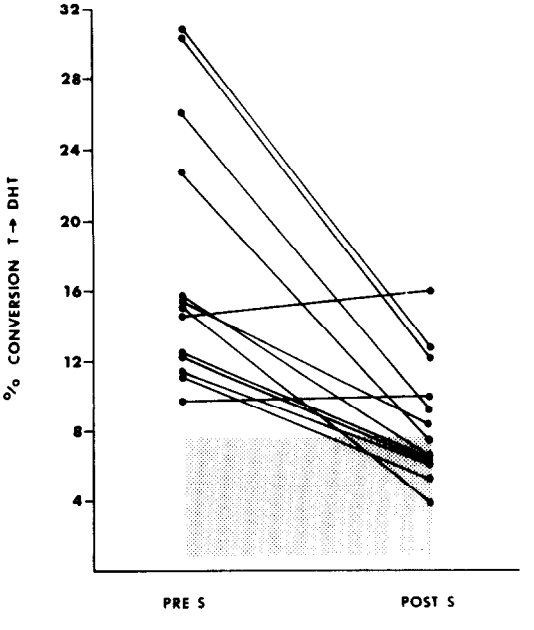


Fig. 2. The percent conversion of T to DHT in hirsute patients before and after 1 month of S. Shaded area represents 95% confidence limits of normal values.

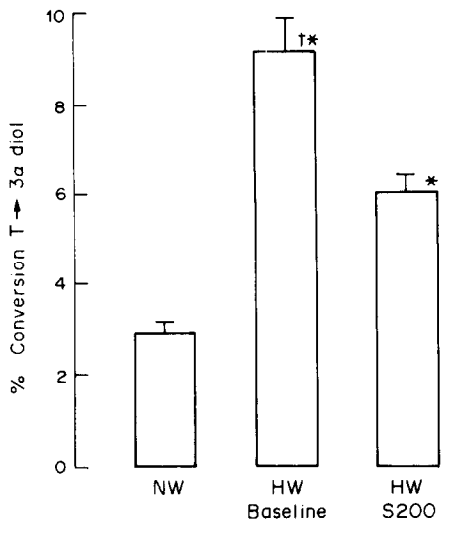


Fig. 3. The percent conversion of T to 3α-diol in normal women (NW), hirsute women (HW) and in HW after S. *Compared to NW (HW Baseline, $P < 0.01$, HW S 200, $P < 0.05$). †HW Baseline vs HW S 200 ($P < 0.05$).

of T to 3α-diol. There were no significant differences in these decrements and no correlations between the changes in 5α-RA and 5α-3α-keto reductase activity.

The repeated incubation of [$^{14}$C]T with the same skin minces resulted in an assay coeficient of variation of 9.03% for the conversion ratio of T to DHT and 8% for the conversion of T to 3α-diol. Values obtained *in vitro* for conversion ratios which fell out of the range of 2 coefficients of variation of the assay were considered significant changes.

The maximum *in vitro* inhibitory effect of S on the conversion of T to DHT occurred with a concentration of $1.2 \times 10^{-5}$ M ($33 \pm 44\%$ decrease (Fig. 4). The decrease in 5α-RA obtained with $1.2 \times 10^{-5}$ M of S per incubation was significantly greater than the effect using $1.5 \times 10^{-6}$ M ($P < 0.05$). This latter concentration also resulted in a significant reduction in the conversion of T to DHT ($P < 0.01$) [Fig. 4].

A significant decrease in the mass of DHT produced was observed with S concentrations of $1.2 \times 10^{-6}$ and $1.2 \times 10^{-7}$ M ($26 \pm 8\%$ and $26 \pm 8.8\%$; $P < 0.02$). The conversion of T to 3α-diol was significantly reduced with doses of $1.2 \times 10^{-5}$ M ($48 \pm 4\%$; $P < 0.001$) and $1.2 \times 10^{-6}$ M ($34 \pm 6\%$; $P < 0.05$) [data not depicted].

The clinical response of HW to S was evaluated after 6 months. As a group, the FG score decreased from $21 \pm 1$ to $14.9 \pm 0.7$, $P < 0.05$. The change in the FG score was +1 (19 to 20) and 0 (14 to 14) in 2 non-responding patients, but the store decreased in the others ranging from −3 to −12 (an average decrease of 30.3%). The correlation between the decrement in 5α-RA and the decrement in the FG score in all patients was of borderline significance, $r = 0.45$, $P > 0.05 < 0.1$.

## DISCUSSION

This study demonstrates that S inhibits genital skin 5α-RA in hirsute women. A substantial decrement of 5α-RA in the genital skin was found after 1 month of S therapy. Eleven of thirteen (84.6%) patients demonstrated a reduction in 5α-RA with S. This figure is comparable to the percentage of hirsute patients showing improvement when treated with S (75–80%) [1, 2, 5–7]. Of interest is the observation that the 2 patients who did not have a reduction in 5α-RA also did not achieve any clinical benefit from S as evidenced by clinical findings after 6 months of therapy.

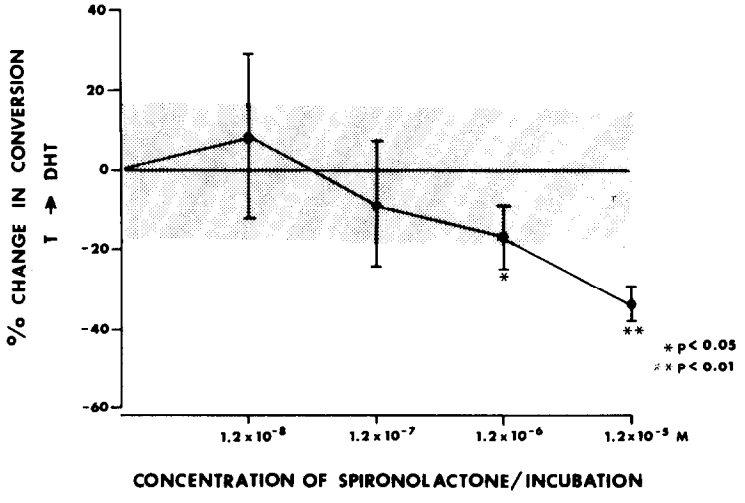


Fig. 4. The percent change of the conversion of T to DHT in genital skin incubated with increasing molar concentrations of spironolactone. The shaded area represents 2 coefficients of variation of the assay for the conversion of T to DHT.

Our *in vitro* studies indicated a dose-related decrement in 5α-RA with significant changes occurring with a S concentration range of $1.2 \times 10^{-7}$ and $1.2 \times 10^{-5}$ M. Although we cannot be sure what tissue levels of S occur with oral ingestion of 200 mg S, we can estimate by calculation that serum levels would be in the range of concentrations where S was demonstrated to have an inhibitory effect on 5α-RA *in vitro*.

The 50% reduction in 5α-RA in this study is comparable with the reduction of 5α-RA observed using CPA [12]. Mowszowicz *et al.* measured 5α-RA in suprapubic skin homogenates before and after treatment with oral CPA and percutaneous estrogen for 1 year [12]. The reduction in 5α-RA was interpreted as being secondary to a decrease in circulating androgen levels as well as in the competition of CPA with T at the receptor level. However, there does not appear to be a close correlation between the decrement in 5α-RA and the change in serum steroid levels.

The reduction in serum androgens, such as T, with S are only modest (20%) after 1 month [3, 5]. This would not be expected to result in the 50% reduction in 5α-RA observed in this study. Furthermore, genital skin is influenced less by circulating steroid levels than is suprapubic skin. Taken together with our *in vitro* evidence, our data would suggest that the effect of S on 5α-RA is through a direct inhibition, and is not mediated through a decrement in serum androgen levels. Although the correlation between the decrements in 5α-RA and FG scores was only of borderline significance, we suggest that the major way in which S exerts a beneficial effect in hirsutism is through an inhibition of peripheral androgen action.

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