Journal of Dermatological Science (2004) **34**, 11—16

Journal of Dermatological SCIENCE

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# Comparative studies on level of androgens in hair and plasma with premature male-pattern baldness

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Received 20 August 2003; received in revised form 18 November 2003; accepted 19 November 2003

**KEYWORDS**
Vertex hair;
Plasma;
Androgenic steroid;
Premature MPB;
GC—MS

**Summary** ***Background:*** It is well known that male-pattern baldness (MPB) is not started from occipital, but frontal or scalp of head. We can assume that distribution of androgenic steroids is different for each region of the head. ***Objective:*** We hypothesize that the levels of androgenic steroids are different not only between vertex hair with MPB and controls but also between occipital hair with MPB and controls. Moreover, we want to search for the biochemical indicator in plasma and hair sample (baldness: 22, non-baldness: 13) obtained from dermatology of medical center. After then, we desire to present fundamental data regarding diagnosis, medical cure, and prevention for premature MPB. ***Methods:*** After hair and plasma were hydrolyzed, and then extracted with organic solvent. To assess androgenic steroids levels, we used gas chromatography—mass spectrometry (GC—MS) system in selected ion monitoring mode. ***Results:*** The level of dihydrotestosterone (DHT) and the ratio of testosterone to epitestosterone (T/E ratio) in vertex hair from premature baldness subjects were higher than in the sample of non-baldness subjects ($P < 0.001$, 0.001), whereas the levels of androgens in occipital hair from the same baldness group were not different. In addition, we discovered the levels of DHT, testosterone, and DHT/T ratio in plasma from premature MPB were higher than in those of control subjects ($P < 0.001$, 0.001, 0.005). ***Conclusion:*** We verified that the distribution of androgenic steroids is unlike in various regions of individual subjects. Moreover, the increased DHT/T ratio in balding plasma indirectly confirms the high activity of 5α-reductase type II.


*Abbreviations:* MPB, male-pattern baldness; SIM, selected ion monitoring; 5α-R II, 5α-reductase type II; DHT, dihydrotestosterone; T, testosterone; T/E ratio, the ratio of testosterone to epitestosterone; GC—MS, gas chromatography—mass spectrometry; MSTFA, *N*-methyl-*N*-trimethylsilyltrifluoroacetamide; DTE, dithioerythritol; Flophemesyl-Cl, pentafluorophenyldimethylsilyl chloride

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0923-1811/$30.00 
doi:10.1016/j.jdermsci.2003.11.005

## 1. Introduction

To date, just a few studies have explored the concern of hair pattern and male-pattern baldness (MPB), which is heritable, androgen-dependent. The hair growth cycle is anagen, catagen, and telogen [1]. As the hair-growth cycle of baldness becomes shorter, the hair follicle gets smaller [2]. Consequently, the hair loss of baldness progresses rapidly and easily. The various hormones affect hair growth, the most studied are the androgens, particularly for MPB. Although the pathogenesis of MPB is not well understood, it is generally accepted that it may be determined by an increase of scalp 5α-reductase type II (5α-R II) activity. The action of androgens is mediated by the androgen receptor, which modulates the transcription of androgen-responsive genes, which play a major role in baldness.

Testosterone, the major circulating androgen in man, can be metabolized to dihydrotestosterone (DHT) by the enzyme steroid 5α-R II in most target organs. Based on affinity of DHT for the androgen receptor, DHT is fivefold more potent than testosterone [3,4]. DHT is implicated in the pathogenesis of several disorders, including benign prostatic hyperplasia, prostate cancer, hirsutism, acne vulgaris, and alopecia [3—5].

On the other hands, epitestosterone, a natural epimer of testosterone, is a physiological constituent of body fluids in many species, including man. It was demonstrated that epitestosterone inhibits the androgenic effect by competition with testosterone for receptor binding sites [6,7]. In vitro experiments with 5α-R II from rat prostate confirmed the earlier observation [6], that epitestosterone is an effective inhibitor of the conversion of testosterone to DHT [7].

It is special status that MPB is started at the temporal region. Within men, results indicate approximately 30% higher levels of total androgenic receptors in frontal hair follicles than those in occipital follicles [8]. Furthermore, the level of 5α-R II increased 60% in frontal area when compared with occipital hair follicles in male baldness [8]. The other author insisted correlation association between prostate cancer and vertex baldness [9]. This association appears to be more evident for high-grade prostate cancer. Moreover, it was discovered that free testosterone in hair is strongly associated with prostate cancer risk and vertex baldness [10]. When we think collectively about the above research, we can assume that distribution of androgenic steroids is different for each region of the head. It is also possible to presume that androgenic steroids in vertex hair may be associated with MPB.

In 1999, we determined androgenic steroids in hair by using gas chromatography—mass spectrometry (GC—MS) [9]. We compared the levels of DHT, epitestosterone, and testosterone in the hair of 19 advanced-stage balding fathers (aged 28—55) and 16 of their sons (aged 8—16) and a control group in 2001 [12].

Perhaps determining hormone serum levels are useful diagnostic measures in hormone-dependent dermatoses [13]. In serum, testosterone and DHT were measured using a radioimmunoassay and then compared levels of those with the balding sample [10]. Using the background knowledge, we quantified androgenic steroids in premature MPB plasma and control groups using GC—MS.

Eventually, we want to search for the biochemical indicators in various biological samples and present fundamental data regarding diagnosis, medical cure, and prevention for premature MPB.

## 2. Materials and methods

### 2.1. Chemicals

Ethanol and acetone were HPLC grade (JT Baker, Phillipsburg, NJ, USA). Testosterone (4-androsten-17β-ol-3-one), epitestosterone (4-androsten-17α-ol-3-one), and dihydrotestosterone (5α-androstan-17β-ol-3-one) were purchased from Sigma—Aldrich (St. Louis, MO, USA). A 6:1 (v/v) mixture of $d_3$-testosterone and $d_3$-epitestosterone, an internal standard, obtained from Cologne Laboratory (Institute of Biochemistry, German Sports University, Germany), was dissolved in methanol to give concentrations of approximately 3 and 0.5 ng/ml, respectively, for accuracy in the testosterone/epitestosterone. To compensate for instrumental variations and losses, we purchased pentafluorophenyldimethylsilyl chloride (Flophemesyl-Cl), *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA), ammonium iodide ($NH_4I$), and dithioerythritol (DTE) from Sigma—Aldrich.

### 2.2. Preparation of standard solution

A stock solution of the three steroids was manufactured at concentrations of 1 mg/ml in methanol. The stock solution was used to prepare a working solution of varying concentrations (0.01—10 ng/ml) in methanol.

### 2.3. Patients

Hair samples and peripheral blood were obtained from 18 male patients aged from 26 to 43 (mean

27.64) with mild to moderate hair loss (loss according to Norwood classification IIIa—V), attending a hair clinic for treatment of male-pattern baldness. Hair was clipped from the vertex and occipital area of each patient. Patients had not been treated with any medication, which can affect the androgen metabolism. The control groups consisted of 13 healthy males aged 25—27 (mean 26.07).

### 2.4. Preparation of hair samples

The subjects were organized into two groups: 18 in the premature balding group and 13 in the non-balding group. The hair was washed with ethanol, distilled water, and acetone. After drying (60 °C), the hair was cut about 1—2 mm. The internal standard solution (10 μl) was added to 50 mg of hair sample. Then 2 ml of 1 M NaOH was added, and the solution was heated at 80 °C for 1 h. After hydrolysis, 1 ml of a phosphate buffer (pH 7.0) was added and the pH was adjusted to 10—11 by adding 2 M HCl. Then liquid—liquid extractions were made with 3 ml of *n*-pentane. The organic layers were then evaporated until they were dry. The residue was dried in a vacuum desiccator over $P_2O_5$/KOH for at least 30 min [11,14].

### 2.5. Preparation of plasma samples

The subjects were organized into two groups: 22 in the premature balding group and 13 in the non-balding group. The internal standard solution (10 μl) was added to 1 ml of a plasma sample. Enzyme hydrolysis was performed using 100 μl of β-glucuronidase at 55 °C for 1 h. Then 1 ml of phosphate buffer (pH 7.0) was added and the pH was adjusted to 10—11 by adding 2 M HCl and two extractions were made with 3 ml of *n*-pentane. The organic layers were then evaporated until they were dry. The residue was dried in a vacuum desiccator over $P_2O_5$/KOH for at least 30 min [11,14].

### 2.6. Derivatization

As derivatization of androgens, the residue was derivatized by the Flophemesyl-Cl (40 μl) at room temperature for 15 min. The excess reagent was evaporated under a stream of nitrogen at 60 °C, and 25 μl of mixture reagent (MSTFA:$NH_4$I:DTE, 1000:4:5, v/w/w) was added. Then the mixture was heated at 60 °C for 15 min. Afterwards, derivatization aliquots (2 μl) were injected into the GC column by an autosampler [11,14].

### 2.7. GC—MS analysis of androgenic steroids

GC—MS analyses both in scan and SIM modes were performed with a HP Model 6890 Plus gas chromatography interfaced to an HP Model 5973 Mass selective detector (Hewlett-Packard, Palo Alto, CA, USA) employing an Ultra-1 (Agilent, cross-linked methyl silicone gum phase) capillary column (25 m × 0.2 mm i.d., 0.11 μm-film thickness). The carrier gas was helium at a column pressure of 121 kPa (column flow: 0.8 ml/min at 280 °C).

Regarding the temperature program, the initial oven temperature was set at 220 °C for 2 min. Then the column was heated to 240 °C at a rate of 4 °C/min. Thereafter the rate was increased to 15 °C/min until 300 °C was reached.

In the selected ion-monitoring (SIM) mode, the peak identification was achieved by comparing the retention times and matching the area ratios of three characteristic ions with those of respective standards.

### 2.8. Statistical analysis

Comparisons between two groups of data were determined by unpaired Student's *t*-test assuming equal variance and two trail populations, and statistical significance was $P < 0.05$.

## 3. Results

### 3.1. The evaluations of DHT (ng/g) and the T/E ratio in the vertex and occipital hair

Our data shows that the levels of DHT and the ratio of testosterone to epitestosterone (T/E ratio) in vertex hair from premature baldness subjects were higher than in that of non-baldness subjects (Fig. 1), whereas the DHT levels and the T/E ratio in occipital hair from the same baldness group were not significantly different. Although the levels of the testosterone in vertex hair was observed no significant difference between the premature balding and the non-balding men, the level of epitestosterone was somewhat lower in the vertex hair of premature baldness subjects than in that of control group.

The concentration median point (Med.), range and mean ± standard deviation (mean ± S.D.) in the vertex hair and occipital hair of baldness and non-baldness were determined by GC—MS with the above extraction method and is described in Tables 1 and 2 .

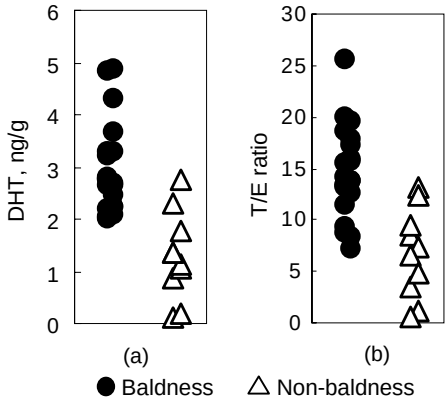


**Fig. 1** The level of DHT (ng/g) and the T/E ratio in vertex hair of premature baldness. (a) DHT and (b) T/E ratio. The amount of DHT was higher in vertex hair of premature baldness than in non-baldness vertex hair ($P < 0.001$). Also, T/E ratio was greater in vertex hair of balding than in control subject ($P < 0.001$). (●) Baldness and (△) non-baldness.

### 3.2. The levels of DHT (ng/ml), testosterone (ng/ml), and DHT/T ratio in plasma

The baldness plasma levels of testosterone and its metabolite, DHT were higher than those of age-matched controls. The balding group revealed amounts of testosterone of 3.19—5.83 ng/ml (mean, 4.36 ng/ml), whereas the levels of testosterone of the control group were 2.06—4.36 ng/ml (mean, 3.05 ng/ml, $P < 0.001$) (Fig. 2a). The concentrations of DHT were shown to be different between the balding group (mean, 71.84 ng/ml; range, 20.50—221.08 ng/ml) and the non-balding subject (mean, 1.41 ng/ml; range, 0.45—2.40 ng/ml, $P < 0.001$) (Fig. 2b). These results correspond with the report of Demark-Wahnefried et al. that elevated testosterone and DHT exists in baldness serum by a radioimmunoassay [10]. In addition, the ratio of DHT/T was significantly greater in baldness plasma than in that of the non-baldness. The

**Table 1** The quantification of steroids (ng/g), T/E ratio, and DHT/T ratio in vertex hair of premature baldness

| | Baldness ($n = 18$) | | | Non-baldness ($n = 13$) | | | $t$-test ($P$-value) |
|---|---|---|---|---|---|---|---|
| | Med. | Range | Mean ± S.D.[b] | Med. | Range | Mean ± S.D. | |
| Steroid[a] | | | | | | | |
| DHT | 2.80 | 1.99—4.88 | 2.94 ± 0.88 | 1.20 | ND[c]—2.74 | 1.25 ± 0.89 | <0.001 |
| Epitestosterone | 0.71 | 0.19—0.97 | 0.71 ± 0.66 | 0.85 | ND[c]—1.60 | 0.82 ± 0.46 | NS[d] |
| Testosterone | 8.33 | 8.11—18.8 | 8.59 ± 3.07 | 8.35 | 0.39—18.6 | 9.12 ± 5.71 | NS[d] |
| T/E ratio | 18.6 | 7.25—25.5 | 18.2 ± 15.6 | 6.50 | ND[c]—13.1 | 6.70 ± 4.41 | <0.001 |
| DHT/T ratio | 0.26 | 0.17—0.59 | 0.26 ± 0.16 | 0.16 | ND[c]—0.50 | 0.16 ± 0.15 | NS[d] |

[a] Analyzed on a ultra-1 capillary column (25 m × 0.2 mm i.d. × 0.33 μm-film thickness; Agilent) in the SIM mode.
[b] S.D., standard deviation for $n = 3$; values in parentheses are relative standard deviations (%).
[c] Not detection.
[d] Not significant.

**Table 2** The quantification of steroids (ng/g), T/E ratio, and DHT/T ratio in occipital hair of premature baldness

| | Baldness ($n = 18$) | | | Non-baldness ($n = 13$) | | | $t$-test ($P$-value) |
|---|---|---|---|---|---|---|---|
| | Med. | Range | Mean ± S.D. | Med. | Range | Mean ± S.D. | |
| Steroid | | | | | | | |
| DHT | 2.98 | 0.39—7.30 | 2.94 ± 1.32 | 1.85 | 1.12—2.81 | 1.88 ± 0.56 | NS |
| Epitestosterone | 0.77 | 0.13—2.52 | 0.71 ± 0.66 | 0.74 | 0.48—1.28 | 0.73 ± 0.29 | NS |
| Testosterone | 8.56 | 4.91—15.74 | 8.59 ± 3.07 | 12.31 | 7.97—17.56 | 12.23 ± 3.13 | NS |
| T/E ratio | 13.42 | 1.00—35.21 | 14.44 ± 7.80 | 14.85 | 8.45—21.70 | 14.40 ± 4.11 | NS |
| DHT/T ratio | 0.38 | 0.11—0.68 | 0.37 ± 0.16 | 0.15 | 0.07—0.24 | 0.16 ± 0.05 | NS |

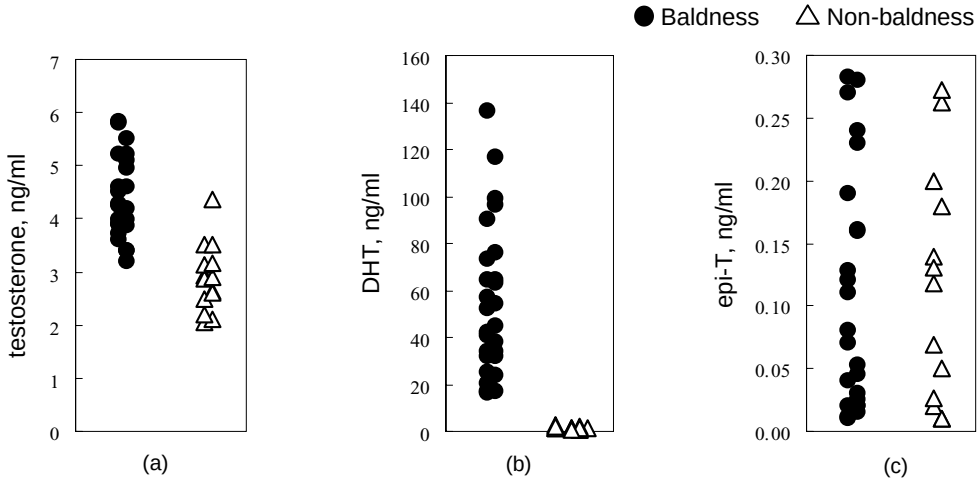


**Fig. 2** The concentrations of testosterone (ng/ml), DHT (ng/ml), and epi-T (ng/ml) in plasma. (a) Testosterone; (b) DHT and (c) epitestosterone. The levels of DHT and testosterone in plasma of premature MPB were significantly higher than those of the non-baldness subjects ($P < 0.001$). There is no difference in the quantification between the two groups in the case of epitestosterone. (a) Baldness: $4.36 \pm 0.76$ (mean $\pm$ S.D.) and non-baldness: $3.05 \pm 0.61$. (b) Baldness: $71.84 \pm 54.15$ and non-baldness: $1.41 \pm 0.56$. (c) Baldness: $0.08 \pm 0.09$ and non-baldness: $0.11 \pm 0.10$. (●) Baldness and (△) non-baldness.

ratio of DHT/T, as an indicator of the activity of the enzyme 5α-R II [15], was on average, 18.54 in the plasma of premature balding subjects and 0.48 ($P < 0.005$) in the plasma of non-balding samples (Fig. 3).

Although levels of testosterone and DHT in plasma have noticeable results, there is no difference in the quantification between the two groups in the case of epitestosterone (Fig. 2c).

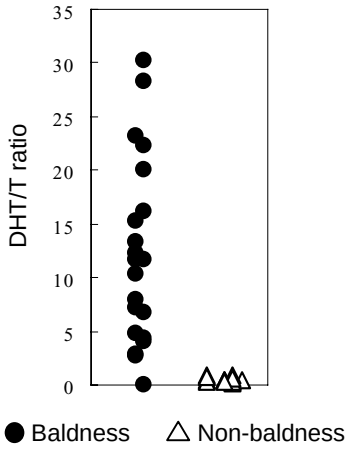


**Fig. 3** The evaluation of DHT/T ratio in plasma. The ratio of DHT/T was significantly higher in plasma of premature MPB than in that of the control ($P < 0.005$). This ratio is an indicator of the 5α-reductase activity. Baldness: $4.36 \pm 0.76$ (mean $\pm$ S.D.) and non-baldness: $3.05 \pm 0.61$.

## 4. Discussion

In our previous study, we found that, high levels of DHT and testosterone, and an amplified ratio of the T/E are characteristics of MPB cases in the advanced-stage hair [12]. The levels of 5α-R II activity and the androgen receptor were higher in the frontal follicles of men with MPB [8]. Base upon previous findings, we collected hair samples as hair region (vertex and occipital hair of premature baldness) and investigated the differences of androgenic steroid as hormone distribution.

Our data show that the level of DHT and the T/E ratio in vertex hair from baldness were higher than in the non-baldness subjects, whereas the DHT level and the T/E ratio in occipital hair from the same baldness were not significantly different between the two groups. Therefore, we demonstrated that the distribution of androgenic steroids is different in the various regions of individual subjects. It is also possible to conclude that MPB is generated by the distribution of androgenic steroids in the vertex.

On the other hands, our data indicate that level of epitestosterone, which inhibits the conversion of testosterone to DHT, was somewhat lower in balding hair samples than in those of control subjects and the T/E ratio was greater in premature baldness hair than in those of the control sample. This result corresponds with the experiment where epitestosterone acts as an antiandrogen in rat prostate [16]. Also, it was demonstrated that epitestosterone blocked the action of testosterone,

presumably in part by a 5α-R II inhibition in an animal tissue experiment [6].

Our present work indicates that the plasma levels of DHT and testosterone were higher in premature baldness samples than in those of age-matched controls. It is close to aspects that have already determined high level of testosterone in baldness serum as a result of a radioimmunoassay [10]. And the saliva testosterone concentration in balding stages was significantly elevated when compared to the non-balding group, according to other group [15]. The significance of the results that the ratio of DHT to testosterone (DHT/T ratio) was greater in the plasma of premature baldness than that in non-baldness samples [17]. The ratio of DHT/T was known as an indicator of the activity of the enzyme 5α-R II [15]. The increased DHT/T ratio in the balding sample indirectly confirmed the high activity of 5α-R II. In case of the levels of epitestosterone were not different between the premature balding group and the non-balding group. We predict that the concentrations of epitestosterone are so little in the plasma that epitestosterone cannot act as an inhibitor of 5α-R II.

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