blood, we estimate that the rate of generation in vivo is likely to be \( \sim 3 \text{ fmol} \cdot \text{L}^{-1} \cdot \text{min}^{-1} \). The blood concentrations of Gc-MAF thus are likely to be quite low; therefore, it is unlikely to act as an endocrine factor. In view of its expected increased generation at sites of inflammation, because of the presence of a large number of T- and B-lymphocytes, it is much more likely to act similarly to a paracrine or autocrine agent.

No in vivo or in vitro assays for Gc-MAF have been reported. The current approach in which Gc-MAF generated by lymphocyte enrichment is then demonstrated by lectin-ELISA may enable investigation into the physiology and pathology of this important compound and its effects on bone remodeling.

References


Dehydroepiandrosterone (DHEA) and Testosterone Concentrations in Human Hair after Chronic DHEA Supplementation, Pascal Kintz,1 Vincent Cirimele,1 Marc Devaux,2 and Bertrand Ludes2 (1 Institut de Médecine Légale, 11 Rue Humann, F-67000 Strasbourg, France; 2 Institut de Médecine Légale, Place Varlet, F-59000 Lille, France; *author for correspondence: fax 33-3-88-24-0085, e-mail pascal.kintz@wanadoo.fr)

Endogenous anabolic steroids and their precursors in the form of dietary supplements have become widely available as over-the-counter tablets in the United States or through the Internet in other countries.

Dehydroepiandrosterone (DHEA) is an endogenous steroid produced by the ovaries and adrenal glands. As a precursor to testosterone and estrogen, DHEA can be converted peripherally to androstenedione, testosterone, and dihydrotestosterone, and aromatized to estrogen.

Recently, athletes have begun taking DHEA, theoretically hoping to derive some competitive benefit from its conversion to testosterone. However, in a few subjects (I, 2), no effect of DHEA on body weight, body mass, resting metabolic rate, total energy expenditure, or protein synthesis was demonstrated. Late in 1996, the International Olympic Committee (IOC) Medical Commission added DHEA to the list of prohibited compounds.

In a study performed by Dehennin et al. (3), labeled DHEA was converted to testosterone, and therefore, some questions were received by our laboratory from sports federations concerning the potential increase of the urinary testosterone/epitestosterone ratio (T/E) through DHEA supplementation. According to Bosy et al. (4), the urinary T/E ratio is only slightly affected for a short period of time (2–5 h), without exceeding 6:1, the current acceptable ratio for the IOC. In their study, seven male volunteers ingested a daily DHEA dose of 50 mg each morning for 30 days. Urine specimens were collected before ingestion and 2–3 h after ingestion. Their urinary baseline T/E ratios were 0.1–1.2. The authors reported that individual postdose T/E ratios ranged from 0.01 to 3.7, as measured each day.

In opposition, in a study by Bowers (5), a 47-year-old subject, with a predose urinary T/E ratio of 2.4, who received single 50-, 100-, or 150-mg doses of DHEA for 4 days at breakfast had a T/E ratio >6:1 for all three doses on day 3, tested on a 24-h sample. The actual T/E ratios were 8.1, 11.4, and 14.4, which can be indicative of exposure to exogenous steroids.

In the current study, we investigated the concentrations of DHEA and testosterone in hair from three subjects who received DHEA for 30 days. Hair is a cumulative specimen that has been demonstrated to document chronic exposure, acting as an historical record. Although not yet recognized by the IOC, hair has demonstrated useful applications in forensic and clinical toxicology.

Three male subjects (30, 38, and 46 years of age; urinary T/E <2) took DHEA (Natrol) at breakfast for 30 consecutive days as a single dose of 25 mg (tablet). A strand of hair was collected before the DHEA was administered to...
determine basal DHEA and testosterone concentrations; another strand was collected 10 days (the time at which the hair emerges from the skin) after the last DHEA dose to determine drug accumulation. Both testosterone and DHEA were analyzed by gas chromatography coupled to mass spectrometry according to our published procedure (6). In each case, a 1-cm section from the root of the hair was analyzed, roughly corresponding to 1 month of hair growth.

Briefly, 100 mg of hair was incubated for 15 min at 95 °C in 1 mL of 1 mol/L NaOH in the presence of 1 ng of testosterone-d_4, used as the internal standard. After cooling, the homogenate was neutralized with 1 mL of 1 mol/L HCl, and 2 mL of 0.2 mol/L phosphate buffer (pH 7.0) was added.

The drugs were extracted by solid-phase extraction. The Isolute C_18 columns were conditioned with 3 mL of methanol, followed by 2 mL of deionized water. After sample addition, the columns were washed twice with 1 mL of deionized water. After column drying, analyte elution occurred with the addition of two 0.75-mL aliquots of methanol. The eluant was evaporated to dryness under nitrogen at 40 °C, and the residue was reconstituted in 1 mL of 0.2 mol/L phosphate buffer (pH 7.0). A further purification step was achieved by addition of 100 mg of Na_2CO_3/NaHCO_3 (1:10, by weight) and 2 mL of pentane. After agitation and centrifugation, the organic phase was removed and evaporated to dryness. The residue was derivatized by the addition of 50 μL of N-methyl-N-trimethylsilyl trifluoroacetamide/NH_4I/2-mercaptoethanol (1000:2.5, by volume), and then incubated for 20 min at 60 °C.

A 4-μL aliquot of the derivatized extract was injected into the column of a Hewlett Packard gas chromatograph (6890 Series) via a Hewlett Packard (model 7673) autosampler. The flow of carrier gas (helium; purity grade N55) through the column (HP5-MS capillary column; 5% phenyl-95% methylsiloxane; 30 m × 0.25 mm i.d.; 0.25-mm film thickness) was 1.0 mL/min. The injector temperature was 270 °C, and splitless injection was used with a split valve off-time of 1.0 min, using the pulsed mode. The column oven temperature was programmed to rise from an initial temperature of 150 °C, maintained for 1 min, to 295 °C at 30 °C/min, and was maintained at 295 °C for the final 8 min. The detector was a Hewlett Packard 5973 operated in the electron impact mode. The electron multiplier voltage was set at 600 V above the electron impact-tune voltage.

Linearity was observed in the range 1–50 pg/mg for both testosterone and DHEA. The relative extraction recoveries were 91.6% and 92.2% for DHEA and testosterone, respectively. The limit of detection was 0.5 pg/mg for both compounds. The within-run imprecision (CV) was 12% for DHEA (at 6.6 pg/mg) and 6.6% for testosterone (at 3.8 pg/mg), respectively (6).

The measured concentrations are reported in Table 1. The basal concentrations in hair were within the physiological ranges we reported previously (6) for both DHEA (1.2–6.7 pg/mg) and testosterone (0.5–9.8 pg/mg). After 30 days of daily DHEA supplementation, the incorporation of DHEA into the hair was obvious, with large interindividual variation, probably because of differences in hair pigmentation. On the other hand, no increase in the testosterone concentration in hair was observed. In this case, DHEA supplementation did not produce increased concentrations of testosterone in hair.

Melanins are responsible for the color of hair, as determined by the quantity of phaeomelanin and eumelanin present in hair. Black and brown hair contain more eumelanin than red and blond hair. Current evidence suggests that melanin is the principal component in the binding of drugs in hair. Several studies performed with animals and humans reported that organic compounds are excreted preferentially in dark hair and suggested that excretion of drug is closely linked to the presence of melanin (7). Our studies suggest that hair color or melanin content may be the major determinant of DHEA binding, and consequently, may produce color bias in hair testing for DHEA.

In conclusion, this study may be interpreted as a useful adjunct to the characterization of DHEA abuse because the drug is easily incorporated into hair. The findings that the testosterone concentration is not increased in hair after repetitive administration of DHEA should not impact the urinary results.

Table 1. DHEA and testosterone concentrations in hair before and after DHEA supplementation. a

<table>
<thead>
<tr>
<th>Subject</th>
<th>DHEA, pg/mg</th>
<th>Testosterone, pg/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>1 (black hair)</td>
<td>4.9</td>
<td>34.8</td>
</tr>
<tr>
<td>2 (blond hair)</td>
<td>4.0</td>
<td>8.6</td>
</tr>
<tr>
<td>3 (dark brown hair)</td>
<td>6.7</td>
<td>35.4</td>
</tr>
</tbody>
</table>

a Subjects received one 25-mg DHEA tablet per day for 30 days.

References