Physiological Concentrations of DHEA in Human Hair

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Abstract

In 1974, steroids were added to the list of doping agents banned by the International Olympic Committee because of their effects on the performance of the athletes. Dehydroepiandrosterone (DHEA) is a steroid hormone naturally produced by the adrenal glands and by the ovaries. DHEA can be converted into other hormones, including estrogen and testosterone. In the United States, DHEA is classified as a nutritional supplement. This is not the case in France, where the drug is listed as a doping agent. As athletes can abuse DHEA to benefit from its conversion to testosterone, there is a need to establish the physiological range of DHEA concentrations in human hair. DHEA was investigated in hair obtained from 27 control subjects, including 15 males and 12 females aged 17–42 years. After decontamination with dichloromethane, 100 mg of hair was incubated in 1 M NaOH in presence of 1 ng of testosterone-d3. After neutralization, the extract was purified using solid-phase extraction with Isolute C18 columns and subsequent liquid–liquid extraction with pentane. After silylation, DHEA was analyzed by gas chromatography–mass spectrometry. Results were linear in the range 1–20 pg/mg. Relative extraction recovery was 91.6% with a limit of detection of 0.5 pg/mg. Concentrations were in the range 1.2–6.7 pg/mg (mean value of 4.3 pg/mg) and 0.5 to 10.6 pg/mg (mean value of 5.3 pg/mg) for the males and females, respectively. Extensive chromatographic procedures (two purification steps by solid-phase and liquid–liquid extraction, combined with injection of 4 μl through the column in pulsed mode) were analytical prerequisites for successful identification of DHEA in hair because of the low target concentrations. This new technology may find useful applications in anabolic abuse control.

Introduction

Dehydroepiandrosterone (DHEA) or prasterone and its sulfated form (DHEA-S) are endogenous steroids secreted by the adrenal cortex. DHEA and DHEA-S interconvert with equilibrium favoring conversion from DHEA-S to DHEA. Although the concentration of DHEA-S circulating in adult men and women exceeds that of any other steroid except cholesterol (DHEA-S levels in adult men are 100–500 times higher than testosterone and 1000–10,000 times higher than estradiol concentrations in women), the function of this steroid in maintaining health had historically received little attention in the scientific community. In recent years, however, there has been considerable research concerning the role of DHEA and DHEA-S in aging. Circulating serum levels of DHEA and DHEA-S decline progressively and markedly with aging (1,2).

In the United States, DHEA is classified as a nutritional supplement and, as such, is readily available to the general population. Moreover, because it does not promote muscle growth, DHEA is not considered an anabolic steroid and is not a controlled substance. However, DHEA is listed on the list of doping agents banned by the International Olympic Committee (IOC).

Recently, athletes have begun taking DHEA, theoretically hoping to derive some competitive benefit from its conversion to testosterone, as the compound is sometimes presented as a precursor of testosterone via androstendione (Figure 1) (3).

Quantitative analysis by gas chromatography coupled with mass spectrometry (GC–MS) demonstrated that androstenedione and etiocholanolone are the most abundant metabolites found

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in urine after a single oral DHEA dose of 50 mg (4). A substantial rise in androsterone for a short period was also mentioned by Kazlauskas (3).

As testosterone administration is banned by the IOC, some questions were received from sport federations concerning the potential increase of the testosterone/epitestosterone ratio (T/E) through DHEA supplementation. In fact, the T/E ratio is only slightly affected for a short period of time (3) and does not exceed 6, which is the currently accepted ratio for the IOC. In a controlled study, after administration of 50 mg of DHEA for 30 consecutive days, the results indicate that the drug has a minimal effect on the T/E ratio and would not be expected to give a positive screen for testosterone abuse (5). However, in the same study (5), one individual who ingested a single 250-mg dose of DHEA experienced a subsequent increase in T/E ratio of 40% relative to the predose value.

Conversion of exogenous DHEA to testosterone is low but was proven to be real by the administration of deuterium-labeled DHEA and the subsequent identification and quantitation of deuterium-labeled testosterone (4). The lack of extensive testosterone generation from DHEA in skeletal muscle was explained by the absence in the muscle of both 17β-hydroxysteroid dehydrogenase and 3β-hydroxysteroid dehydrogenase, two essential enzymes for the conversion of DHEA to active androgens (6,7).

In a normal population, the DHEA supplementation dosage is generally in the range of 25–50 mg/day. The dose recommended by supplement suppliers catering to body-building clientele on the Internet is up to 1000 mg/day.

Although not yet recognized by the IOC, hair was recently used to document forensic cases of doping, such as during the cycling Tour de France 1998. As a result, research demonstrating that hair analysis may be a useful adjunct to conventional urine testing in sports has been published recently (8–10).

Because of the lack of suitable reference, the aim of this study was to establish the normal quantitative range of physiological concentrations of DHEA in both male and female human hair for nonathletes.

### Experimental

#### Human hair samples

Full-length hair samples were taken at the surface of the skin from the vertex of 15 male and 12 female volunteers for DHEA testing. Subjects were aged from 17 to 42 years. None of the subjects were professional athletes, and all denied use of doping agents, irrespective of the pharmacological class. All subjects were French, and DHEA is not available in France.

All the hair samples were stored in plastic tubes at room temperature.

#### Chemicals

Dichloromethane, pentane, and methanol were high-performance liquid chromatography grade (Merck, Darmstadt, Germany). All other chemicals were of analytical grade and provided by Merck. DHEA, testosterone, and testosterone-d3 were purchased from...
Sigma (Saint-Quentin Fallavier, France). N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA), 2-mercaptoethanol, and ammonium iodide (NH₄I) were purchased from Fluka (Saint-Quentin Fallavier, France). Isolute C₁₈ columns were purchased from Touzart et Matignon (Courtaboeuf, France). Synthetic melanin was purchased from Sigma (Saint-Quentin Fallavier, France).

Sample extraction for DHEA

Before analysis, samples were decontaminated twice for 2 min using 5 mL of methylene chloride at room temperature.

A 4-cm segment measured from the root was used for the analysis.

One-hundred milligrams of hair was incubated in 1 mL 1M NaOH in the presence of 1 ng of testosterone-d₃ (prepared in methanol and used as internal standard) for 15 min at 95°C. After cooling, the homogenate was neutralized with 1 mL 1M HCl, and 2 mL of 0.2M phosphate buffer (pH 7.0) were added.

The Isolute C₁₈ 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 aliquots of 0.75 mL of methanol. The eluant was evaporated to dryness under nitrogen flow at 40°C, and the residue reconstituted in 1 mL of 0.2M phosphate buffer (pH 7.0). A further purification step was achieved by addition of 100 mg of Na₂CO₃/NaHCO₃ (1:10, w/w) and 2 mL of pentane. After agitation and centrifugation, the organic phase was removed and evaporated to dryness.

The residue was derivatized by adding 50 μL MSTFA/NH₄I/2-mercaptoethanol (1000:2:5, v/v/v), then incubated for 20 min at 60°C.

GC-MS procedure

A 4-μL aliquot of the derivatized extract was injected into the column of a Hewlett Packard (Palo Alto, CA) GC (6890 series) via a Hewlett Packard (7673) autosampler. The flow of carrier gas (helium, purity grade N55) through the column (HP5-MS capillary column, 5% phenyl-95% methyl-siloxane, 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 employed 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 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 EI-tune voltage.

Method validation

A standard calibration curve was obtained by adding 0.1 (1 pg/mg), 0.2 (2 pg/mg), 0.5 (5 pg/mg), 1.0 (10 pg/mg), and 2.0 (20 pg/mg) ng of DHEA to 50 mg of synthetic melanin in suspension in 1 mL 1M NaOH. This procedure was necessary because it was not possible to find hair specimens free of DHEA. However, it must be taken into account that melanin does not provide sufficient control for the effect that other hair constituents play in the uptake, retention, and extraction release of DHEA.

Within-run precision for DHEA was determined using hair that was obtained from a laboratory volunteer and previously pulverized in a ball mill. Relative extraction recovery was determined for DHEA by comparing the representative peak of extracted 50 mg melanin preparation (spiked with a final 5 pg/mg concentration) with the peak area of a metanolic standard at 5 pg/mg. The detection limit (LOD) was evaluated with decreasing concentrations of DHEA spiked in melanin until a response equivalent to three times the background noise was observed.

Analytes were identified and quantitated on the basis of a
comparison of retention times and the relative abundance of three ions with the deuterated internal standard.

Results and Discussion

Table I shows the retention times and the ions monitored for the analytes.

Responses for DHEA were linear in the range of 1–20 pg/mg with a correlation coefficient of 0.9972.

The within-run precision was 11.8%, as determined by analyzing eight pulverized replicates of 100 mg of hair obtained from the same subject and evaluated to contain DHEA at 6.6 pg/mg. The extraction recovery (n = 3) was determined to be 91.6%. The limit of detection of DHEA was 0.5 pg/mg. Extensive chromatographic procedures (two purification steps by solid-phase and liquid–liquid extraction, combined with injection of 4 µL through the column in pulsed mode) were analytical prerequisites for successful identification of DHEA in hair because of the low target concentrations.

Under the chromatographic conditions used, there was no interference with the analytes by any extractable endogenous materials present in hair. The derivatized compounds were found stable for at least one week. Figures 2 and 3 show a typical ion chromatogram using ions m/z 432 and 435 after extraction of two hair specimens obtained from a male and a female, respectively. In the same run, it was possible to identify testosterone and epitestosterone. However, only testosterone was quantitated according to our previous procedure (10).

Twenty-seven specimens obtained from male (n = 15) and female (n = 12) volunteers were analyzed. Individual results are presented in Table II. Concentrations were in the range 1.2 to 6.7 pg/mg, with a mean value of 4.3 pg/mg, and 0.5 to 10.6 pg/mg, with a mean value of 5.3 pg/mg for the males and females, respectively. The studied population was not large enough to conclude that DHEA concentrations are higher in females than in males. All specimens from males tested positive for testosterone at concentrations ranging from 0.5 to 9.8 pg/mg with a mean value of 2.7 pg/mg. The latter concentration is lower than the mean value that was reported previously (3.8 pg/mg) by this laboratory (10). As expected, testosterone concentrations were low in hair obtained from females.

These physiological DHEA concentrations were obtained for nonathletes. However, it is unknown whether athletes participating in high-level sport activity (such as Olympic athletes) would have similar hair concentrations of DHEA. In the past, it has been demonstrated that plasma DHEA concentrations were elevated during exercise in professional soccer players (11). It is therefore possible that continual elevated plasma concentrations of the steroid hormone in response to exercise may result in increased baseline concentrations of the hormone in hair. Until such studies are performed, “normal ranges” determined in nonathletes should be applied very cautiously to the interpretation of hormone concentrations in athlete hair.

Before establishing cutoff concentrations for determining abuse, a more complete epidemiological study must be achieved. It was demonstrated by Field et al. (12) that smoking, obesity, or alcohol consumption have some effect on the “normal ranges” in serum established for DHEA and DHEA-S.

Surprisingly, the mean concentration of DHEA is near to the mean concentration of testosterone in male subjects, although the serum concentrations of DHEA are 100–500 times higher (5) than the concentrations of testosterone. No satisfactory explanation was found to document this finding, as the chemical structures of both compounds are close. Perhaps formation of other metabolic products, such as estradiol or other androgens, and their deposition into hair, might explain this. Additional experiments are needed to document anabolic drug deposition in hair.

It was not possible to establish a correlation between the concentrations of DHEA and the concentrations of testosterone in the male subjects. The correlation coefficient was

$$r = 0.029 \text{ with } p = 0.9181 \text{ (F-test).}$$

Because of the small population that was screened for DHEA, no attempt to correlate DHEA concentration in hair with the corresponding age of the subject was achieved.

Despite interconversion of DHEA and DHEA-S, about 90% of this steroid is in the DHEA-S form. DHEA-S is cleared from the blood much more slowly than is DHEA. Presumably, hair concentrations of DHEA-S would also be higher. This has to be confirmed in further studies.

<table>
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<tr>
<th>Subject</th>
<th>Gender</th>
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<th>Testosterone (pg/mg)</th>
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* Abbreviations: M, male; F, female; ND, under the limit of detection (0.5 pg/mg).
A controlled study, involving three subjects receiving 25 mg of DHEA each day for 30 consecutive days, is currently under investigation in this laboratory to determine the impact of this supplementation on the hair levels of both DHEA and testosterone.

In conclusion, the proposed method seems to be suitable for the evaluation of physiological concentrations of DHEA in both male and female hair specimens. In the same run, testosterone can be quantitated.

References


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