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Technical Briefs

Oral Dehydroepiandrosterone Supplementation Can Increase the Testosterone/Epitestosterone Ratio

Larry D. Bowers[a]

The availability of endogenous anabolic steroids and their precursors in the form of "dietary supplements" has become widespread in the United States. The popularity of dehydroepiandrosterone (DHEA) arose from a number of reports in the popular press that suggested that the results of a study conducted by Morales et al. (1) characterized DHEA as similar to the mythical fountain of youth. Increased use of DHEA among athletes was reported anecdotally before and during the 1996 Olympic Games, and the International Olympic Committee (IOC) Medical Commission explicitly added the compound to the list of prohibited compounds in December 1996, although it would have been considered prohibited previously under the "related compounds" provision of the list. Other groups testing for steroids have either added DHEA as an example of a prohibited compound or assumed that it did not play a role in their testing program.

Some researchers have questioned whether the use of compounds that are precursors of the anabolic steroid testosterone increase testosterone and therefore impact the testosterone/epitestosterone (T/E) ratio. Two studies have reported that no increase in the T/E ratio occurred when DHEA was given (2)(3). We report here on the impact of administration of two over-the-counter DHEA preparations on the excretion of several steroids, and a greater than 6:1 dose-dependent increase of the T/E ratio in one individual.

Two over-the-counter preparations of DHEA were obtained from a health food store and from a pharmacy. Nature's Pride "DHEA 50 mg+" (product A; Nature's Products, Davie, FL) capsules contained DHEA (50 mg), suma (*Pfaffia paniculata*, 25 mg), Korean ginseng (*Panax ginseng*, 25 mg), muira pauma (*Ptychopetalum olacoides*, 25 mg), shitake mushroom concentrate (*Lentinus edodes*, 15 mg), and green tea extract (*Camelia sinesis*, 5 mg). YourLife DHEA tablets (product B; Leiner Health Products, Inc., Carson, CA) contained DHEA (25 mg) as the only active substance documented on the label. Because these products are marketed as natural dietary supplements, the manufacturers are not legally required to comply with the truth in labeling regulations. No testosterone was detected in either preparation by gas chromatography–mass spectrometry (GC-MS) analysis of the capsules or tablets.
Androsterone, etiocholanolone, 11ß-hydroxyandrosterone, 11ß-hydroxyetiocholanolone, androst-5-en-3,17-diol, 5α-androstan-3α,17ß-diol, 5ß-androstan-3α,17ß-diol, dihydrotestosterone, DHEA, T, and E were purchased from either Sigma Chemical Co. or Steraloids and were used as received. The d_3-testosterone and d_3-epitestosterone were synthesized in our laboratory (4). All solvents were high purity (Burdick and Jackson).

The urine samples were analyzed by GC-MS, using a modification of a procedure reported earlier (5). To summarize, 2 mL of urine, to which 90 µg/L d_3-testosterone, 15 µg/L d_3-epitestosterone, and 50 µg/L of methyltestosterone were added as internal standards, was loaded onto a preconditioned C_{18} solid-phase

extraction cartridge, washed with water, and eluted with methanol. The samples were hydrolyzed at pH 7 with ß-glucuronidase (*Escherichia coli*; Boehringer Mannheim Diagnostics) for 3 h at 37 °C. The hydrolysate was extracted with hexane, the extract was dried, and the tetramethylsilyl (TMS)-ether-TMS-enol-ester derivative was formed. Quantitative analysis was performed using selected ion monitoring on a Hewlett Packard model 5970 GC-MS detector. A single point calibration was made for DHEA, androsterone, etiocholanolone, 11ß-hydroxy-androsterone, 11ß-hydroxyetiocholanolone, 5-androsten-3,17-diol, 5α-androstan-3α,17ß-diol, and 5ß-androstan-3α,17ß-diol, using methyltestosterone as the internal standards. For T and E, I constructed a four-point calibration curve by keeping the amount of E constant (10 µg/L) and varying the amount of T. In the latter case, the two coeluting deuterated compounds were used as the internal standards.

Four male subjects taking one of the supplements containing DHEA provided urine samples for the study. All studies were in accordance with the Helsinki Declaration of 1975, and all subjects gave informed consent. The ages of the subjects varied substantially. Subject 1 took both preparations at three dosage levels at different times over a 6-month interval. Subjects 1, 2, and 3 took DHEA for 4 days as a single dose taken at breakfast. Subject 4 took two doses of DHEA. Pre-dose random urine samples were collected from all subjects before the DHEA was taken. On day 3, a 24-h urine sample was collected. On day 4, spot urine samples were collected in the morning and the afternoon.

The use of "endogenous" steroids to enhance performance in sports is becoming more widespread. The reasons for using endogenous substances vary, but include the belief that because a substance is "natural", it is not dangerous, is not prohibited, or cannot be as readily detected by drug-testing protocols. Several studies have been published in which replacement doses of DHEA were given to subjects >45 years of age (1). The recommended replacement dose was 25–50 mg/day. No studies have been carried out on young adults in whom adrenal DHEA production is substantial. The dose recommended by supplement suppliers catering to body-building clientele on the Internet is up to 1000 mg/day. A controversial question in athletic drug testing is whether DHEA is detected in steroid-testing procedures, specifically by increases in the T/E ratio.

As shown in Table 1✦, one of the four volunteers showed a dose-dependent increase in the T/E ratio on days 3 and 4. At doses of 50, 100, and 150 mg/ day, subject 1 had T/E ratios >6. A ratio exceeding 6:1 is used by a number of organizations, including the IOC and the US military, as an indication that additional studies are required to rule out the exogenous use of physiological steroids. The use of two preparations and the absence of any T in either of the DHEA dosage forms clearly indicates that the increase observed here is attributable to administration of DHEA. Given the fact that DHEA can be metabolized to T, the results here are not surprising. In all subjects, the T/creatinine ratio increased substantially, indicating conversion of DHEA to T. This agrees with earlier reports by Mahesh and Greenblatt (6) and Dehennin et al. (3), who showed that a small amount of DHEA (~1.5%) is converted into T. It should be pointed out, however, that the urine excretion may not reflect bioactive T. In subject 1 and 3, the increase in T excretion did not parallel E excretion, and thus the T/E ratio increased. In subject 3, the ratio was less than that which would be considered a violation under the rules of sports. Thus, DHEA could be considered a pro-drug for the production of T.

All subjects showed substantial increases in most endogenous steroid concentrations measured. As summarized in Table 1✦, the excretion rates of androsterone and etiocholanolone increased 50-fold. Interestingly, the 5α/5ß diol ratio changed substantially after 4 days of DHEA administration, but had not changed at prior data points. Neither of these markers is specific for DHEA administration, however. Dehennin et al. (3) reported that DHEA administration could be detected when DHEA glucuronide in the urine was present at concentrations >300 µg/L. In a small study in our laboratory involving nine women and seven men who were not taking DHEA or any other steroid, the DHEA glucuronide concentration was <90

µg/L [women, 25 ± 26 µg/L (mean ± 1 SD); range, 6–70 µg/L; men, 50 ± 27 µg/L; range, 30–90 µg/L]. During the administration studies described, the DHEA glucuronide concentration was >300 µg/L in only one of the six studies involving one of the four volunteers (see Table 1). Other metabolites of DHEA, such as androst-5-en-3ß,17ß-diol, androst-5-en-3ß,17α-diol, androst-5-en-3ß,7α-diol-17-one, and androst-5-en-3ß,16α-diol-17-one, or some ratio of these metabolites may provide a more accurate retrospective record of use.

A recent publication (2) reported that in a study of 12 men in the military, no subject taking 25 mg of DHEA per day had a T/E ratio >6:1. Dehennin et al. (3) reported recently that a single oral dose of 50 mg of DHEA did not increase the T/E ratio in a group of nine healthy males. On the basis of these reports and the data reported here, an increased T/E ratio is unusual, but possible. Pharmacogenetics have a clear role in the disposition of supplemental DHEA. It should also be pointed out that the doses given in the studies mentioned do not come close to the doses taken by some athletes. The IOC specifically prohibited the use of DHEA as a supplement in November 1996. A T/E ratio >6:1, whatever compound is ingested, has been deemed a violation of antidoping rules if a physiological explanation cannot be found. The US military has not banned the use of DHEA, but continues to use the T/E ratio of >6:1 as an indication of T abuse.
Manipulation of the steroid endocrine system for improved athletic performance or appearance has a number of drawbacks, including the potential for failing a drug test. The T/E ratio is an indicator of alterations in steroid metabolism, not a specific marker for exogenous T administration. A number of other compounds, including androstenedione and androstenediol, have been advertised on the Internet as muscle-building supplements and specifically refer to their ability to increase circulating T concentrations. One would expect that these compounds might increase various urinary steroid concentration ratios as well. Androstenedione was added to the IOC prohibited substance list in December 1997. The IOC rules clearly state that related compounds are prohibited, and the close structural similarity of these steroids certainly would place them in this category. Information on the Internet correctly states that these substances are "legal" with respect to the criminal justice system. They are prohibited, however, by the IOC, the National Collegiate Athletic Association, and the National Football League. Given the philosophy expressed in the Dietary Supplement Health and Education Act of 1994, it is unwise to conclude that natural and safe are equivalent or that the US government has established the safety of any dietary supplements.

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