The Effect of Oral Dehydroepiandrosterone (DHEA) on the Urine Testosterone/Epitestosterone (T/E) Ratio in Human Male Volunteers*

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Abstract

Dehydroepiandrosterone (DHEA) is an endogenous androgenic steroid produced by the ovaries and adrenal glands. Research suggests that DHEA can be converted into testosterone in peripheral tissues. Classified as a nutritional supplement, this compound may be purchased without a prescription. The military and international sports organizations prohibit the use of exogenous androgenic/anabolic steroids. Steroid-screening results are considered “positive” when the urinary ratio of testosterone to epitestosterone (T/E), an inactive synthetic byproduct, exceeds 6:1. Human volunteers ingested the recommended daily dose of 50 mg each morning for 30 days to determine if DHEA causes an adverse effect on this ratio. Urinary samples were collected before ingestion and 2–3 h after ingestion. Urine samples were extracted using solid-phase columns and analyzed using a previously developed gas chromatography–mass spectrometry method. T/E results were compared to an average baseline generated from three urine samples obtained before the study. Mean baseline T/E ratios averaged 0.67 for the seven subjects (range 0.1–1.2). The mean T/E ratio after DHEA ingestion ranged from 0.03 to 2.11. Individual postdose T/E ratios ranged from 0.01 to 3.7. The results from these individuals indicate that the administration of DHEA at this dose, for this period of time, has a minimal effect on urine T/E ratios and would not be expected to result in a positive screen for testosterone abuse. One subject agreed to take a single dose of 250 mg. This acute, high dose caused his T/E ratio to increase by 40% relative to the predose value.

Introduction

Controlling steroid abuse requires an effective means to determine the administration of banned substances. For synthetic anabolic androgenic steroids, the identification of the parent steroid and metabolites in urine is evidence that abuse has taken place (1,2). For substances that are produced naturally, like testosterone, the mere presence of the substance in the urine obviously cannot constitute proof of an offense. The notion of setting a concentration cutoff for urinary testosterone was abandoned because the range of values in normal subjects varies widely. A more realistic approach was developed in the early 1980s (3,4): measuring the ratio of testosterone to epitestosterone, the inactive 17-α epimer of testosterone produced as a by-product of testosterone synthesis. The T/E ratio is approximately 1 in the majority of normal males. Exogenous testosterone administration results in an increase in the testosterone/epitestosterone (T/E) ratio because exogenous testosterone is not incorporated into the manufacture of epitestosterone. Additionally, exogenous administration of some of the other anabolic steroids will affect this ratio through a negative feedback mechanism, which decreases testosterone production without affecting epitestosterone production (5). In recognition of this situation, the International Olympic Committee (IOC) and many national and international sport authorities have worded their rules to state that testosterone administration is banned and that the T/E ratio may not exceed 6 (6,7). This is also the criterion currently accepted by the Department of Defense in determining steroid abuse cases.

This study was conducted in response to questions received from military criminal investigation offices concerning dehydroepiandrosterone (DHEA) consumption and its relevance to the investigation of steroid-abuse cases. Use of selected anabolic, androgenic steroids is illegal for service members. DHEA is classified as a nutritional supplement and as such is readily available to the general population. Recently, athletes have begun taking DHEA, theoretically hoping to derive some competitive benefit from its conversion to testosterone despite previous scientific evidence to the contrary. Moreover, individuals in a position to have their urine tested for steroids have begun claiming that elevated T/E ratios are the result of innocent DHEA supplementation. Current preliminary evidence indicates that DHEA may indeed result in T/E ratios approaching 6. The military and drug-testing communities have since stopped reporting the T/E ratio until these issues are resolved.

This study will contribute to the body of knowledge on this relatively new supplement and assist in the litigation of cases in which this drug has been implicated as responsible for abnormal test results in anabolic steroid-abuse cases.

*The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the Department of the Air Force, the Department of the Army, the Department of the Navy, or the Department of the Defense.
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Experimental

Materials
DHEA capsules (50 mg) were obtained from The Vitamin Shoppe (North Bergen, NJ). Testosterone, epitestosterone, 16-α-OH-testosterone, and DHEA standards were purchased from Sigma Chemical Co. (St. Louis, MO). N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) and β-glucuronidase (from E. coli) were also purchased from Sigma Chemical Co. CLEAN SCREEN solid-phase extraction columns (ZSDAU020) were purchased from United Chemical Technologies, Inc. (Bristol, PA). All other chemicals were purchased from Sigma Chemical Co. Solvents were HPLC grade, purchased from Fischer Scientific Products/Fischer Scientific (Fair Lawn, New Jersey).

Subjects, dosing, and urine collection
Seven healthy, male volunteers participated in the study. Demographic information is provided in Table I. The study was conducted under the guidelines and approval of the Institutional Review Board, Medical College of Virginia (Richmond, VA), and each volunteer gave informed consent. Participants submitted three urine samples taken on 3 separate days for baseline determination of their T/E ratios. The study protocol then instructed the participants to collect a urine sample each morning before administration of a 50-mg capsule of DHEA and a urine sample approximately 3 h post-dose (with no voiding after administration of the DHEA capsule until the 3-h collection) for 30 consecutive days. Results of studies performed previously in this laboratory show peak urine levels of DHEA occur approximately 2 h post-dose. This suggests that peak plasma levels occur some time before that, and the 3-h measure of urine T and E was chosen in an attempt to maximize the urine T generated from the dose of DHEA. To determine the effect of an acute high dose of DHEA, one subject agreed to take a single 250-mg dose. This single high dose was given to determine the changes such a dose would have on the T/E ratio measured over a 24-h period.

Extraction and derivatization
Urine samples were used for solid-phase extraction using a modified protocol from Donike et al. (8,9). Five milliliters of urine was pipetted into 16 × 100 mm borosilicate glass test tubes. A six-point standard curve was run with each assay. One microliter was injected for gas chromatography (GC) injection vials. One microliter was injected for gas chromatographic–mass spectrometric (GC–MS) analysis.

Instrumentation
GC–MS analysis was performed using a 5890 GC (Hewlett-Packard, Palo Alto, CA) interfaced to an HP 5972 MS. The GC was equipped with a DB-1MS, fused-silica cross-linked methyl silicone capillary column (15 m × 0.25-mm i.d., 0.25-μm film-thickness, J&W Scientific, Folsom, CA) with helium (1 mL/min) as the carrier gas. Splitless injections were performed with the GC oven temperature programmed at 150°C (held 1 min) to 225°C at 2°C/min. The final ramp was to 300°C at 50°C/min and held for 3 min. The injector and MS temperature was 280°C. The MS was operated in the selected ion monitoring (SIM) mode, and ions 432, 417, (T, E, DHEA), and 520 (internal standard) were collected. A six-point standard curve was run with each assay. Quantitation was determined comparing the integrated peak areas of a sample with that of the standard curve. The integrated peak area of the 432 ion was used for all quantitations. Concentrations of both T and E were varied to generate the standard curve.

Table I. Demographics of Subjects and Results of the Effect of DHEA on Baseline T/E Ratios

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (lbs.)</th>
<th>Weight (lbs.)</th>
<th>Baseline T/E (Mean)</th>
<th>Mean T/E (DHEA)</th>
<th>T/E Range (DHEA)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38</td>
<td>180</td>
<td>1.03</td>
<td>1.10</td>
<td>0.53–2.61</td>
<td>0.40</td>
</tr>
<tr>
<td>2</td>
<td>39</td>
<td>222</td>
<td>0.34</td>
<td>0.43</td>
<td>0.16–1.02</td>
<td>0.18</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>192</td>
<td>0.10</td>
<td>0.03</td>
<td>0.00–0.06</td>
<td>0.01</td>
</tr>
<tr>
<td>4</td>
<td>37</td>
<td>152</td>
<td>0.56</td>
<td>0.98</td>
<td>0.53–1.43</td>
<td>0.25</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>210</td>
<td>0.90</td>
<td>1.68</td>
<td>0.90–3.00</td>
<td>0.52</td>
</tr>
<tr>
<td>6</td>
<td>49</td>
<td>175</td>
<td>1.2</td>
<td>2.11</td>
<td>1.0–3.7</td>
<td>0.51</td>
</tr>
<tr>
<td>7</td>
<td>32</td>
<td>201</td>
<td>0.56</td>
<td>0.77</td>
<td>0.39–1.2</td>
<td>0.18</td>
</tr>
</tbody>
</table>

* Two-tailed t-VALUE α = 0.025; df = 6) = 3.143; |t| = 0.05.

Statistical analyses
The null hypothesis states the T/E ratio would be unaffected by the administration of DHEA. Because it was possible that the T/E ratios could increase or decrease, pre- and post-dosing ratio means were compared using a two-tailed, paired t-test. Significant differences were reported for p < 0.025. The two-tailed t-value (α = 0.025, df = 6) for significance was 3.143.
Results

To verify the accuracy of package labeling for the DHEA used in this study, a random selection of capsules was dissolved in deionized water, and the drug was extracted. The results, as compared with a reference standard, indicated that the package labeling was factual regarding purity and amount.

Quantitations were achieved by comparing integrated peak areas of the samples with the integrated peak areas of the standard curve. Baseline mean T/E ratios ranged from a low of 0.1 to a high of 1.2. Urine samples used to calculate these values were collected on three separate days and exhibited minimal variability (data not shown).

Representative pre- and post-dose chromatograms are seen in Figures 1 and 2, respectively. DHEA pre-dose amounts are significantly lower than the post-dose concentrations. These samples are from approximately halfway through the treatment regimen and depict the relative increase in abundance as a result of one 50-mg capsule. This increase was consistent across test subjects and varied little during the course of the treatment.

T/E ratios after 30 daily doses of 50 mg DHEA are shown in Figure 3. Most subjects showed an immediate increase in T/E ratios after the first day of treatment. Over the 30-day period, the T/E ratios exhibited varying degrees of fluctuation. For example, subject 2 had a baseline mean of 0.34 and a treatment mean of 0.43; in contrast, the baseline mean of subject 6 was 1.20, whereas his treatment mean almost doubled to 2.11. These two subjects marked the least and greatest variations, respectively, observed between the baseline and treatment mean. Subject 6 also showed the greatest difference between his baseline mean of 1.2 and peak treatment ratio of 3.7. All subjects, except subject 3, exhibited a peak T/E ratio at least double their baseline mean at some point during the study. However, t-test results indicated that the difference between the baseline means and the treatment means were not significant (two-tailed t-value \( t = 3.143; df = 6 \); \( p = 0.05 \)).

No peak T/E ratios approached the 6:1 threshold that suggests testosterone abuse. Additionally, all urine testosterone concentrations examined in this study were below 120 ng/mL (data not shown). No adjustment was made to any sample correcting for urinary specific gravity or creatinine concentrations.

The results of a single 250-mg dose of DHEA
are shown in Figure 4. This dose resulted in a 40% increase in the subject's T/E ratio relative to the pre-dose value. The peak T/E ratio after this 250 mg single dose was 1.2, which, again, is much lower than the 6.0 traditionally used as the cutoff indicating testosterone abuse. This increase in T/E ratio occurred 2-h post-dose and persisted until the fifth hour. The ratio returned to pre-dose levels approximately 7-h post-dose. As in the initial study, the urinary testosterone concentration did not surpass 120 ng/mL at any time point tested.

Discussion

DHEA is an endogenous, steroid that is also produced in a sulfated form (DHEA-S) (10). Production of both of these compounds occurs primarily in the adrenal glands. Normal concentrations in adults exceed 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. DHEA and DHEA-S interconvert, with the equilibrium favoring conversion from DHEA-S to DHEA.

Although neither of these compounds has intrinsic androgenic, estrogenic or other classical hormonal activity, both, especially DHEA-S, are converted into androgens and estrogens in peripheral tissues (11). Approximately 50% of total androgens in men are derived from these compounds. In women, 75% of estrogen formation is from DHEA/DHEA-S before menopause, and it rises to almost 100% after menopause.

It has been well demonstrated that the biosynthesis of androgens from DHEA is limited to the appropriate target tissues without leakage of significant amounts of active androgens into the circulation (11). This local biosynthesis and action of androgens, termed "intracrinology", eliminates the inappropriate exposure of other tissues to androgens, thus minimizing the risks of undesirable side effects. Conversion of DHEA occurs primarily in the "classic" steroidogenic tissues (placenta, adrenal cortex, ovary, and testis) and such peripheral tissues as skin, adipose tissue, breast, lung, endometrium, prostate, liver, epididymis, and brain. Two essential enzymes for the conversion of DHEA to active androgens/estrogens are 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase (3). Neither of these enzymes is present in skeletal muscle (12). Without the expression of both these enzymes, it is difficult to conclude that this tissue has the capability of generating testosterone from either DHEA or DHEA-S. This being the case, the use of high doses of DHEA, while having presumed effects in those tissues that possess the enzymatic activity, would not result in the production of testosterone in muscle.

As stated, no evidence exists that DHEA promotes muscle growth. Therefore, the Drug Enforcement Administration had determined DHEA is not an anabolic steroid and is not subject to regulation under the Controlled Substances Act. According to the Federal Food, Drug and Cosmetic Act (FFDCA, Sect. 201), a "dietary supplement" contains one or more of the following: "...a vitamin, a mineral, an herb/botanical, an amino acid, a dietary substance used to increase intake and/or a concentrate, metabolite, constituent or extract". DHEA falls under this provision as long as therapeutic claims are not made, and in 1994, the Dietary Supplement and Education Act opened the door to over-the-counter distribution of DHEA.

The fame, and often fortune, that results from participation in national and international sporting events is a powerful psychological and economic motivator. The pressure to succeed has forced athletes to look for ways to enhance their performance. The first documented case of athletes using performance-enhancing compounds was by the Russian weightlifting team in the 1950s. The compound they used turned out to be testosterone (4). Although testosterone does have androgenic (masculinizing) activity, it is the anabolic (muscle building/reduction in muscle loss) effects that are exploited by athletes.

Beginning in 1972, the IOC instituted full-scale urine drug testing at the Munich Olympic Games. The IOC has banned the use of synthetic anabolic steroids since 1974 and banned the use of exogenous testosterone in 1984. However, because testosterone is an endogenous product, another means of detection was required to determine "abuse". In 1984, the IOC and other sports federations agreed to use the method of Donike (6) to measure T/E ratios. They agreed that a ratio greater than 6:1 would be an indicator of abuse.

Since the appearance of DHEA as an over-the-counter product, investigators have reported that steroid abusers with positive urine drug tests are claiming that their use of DHEA, not exogenous testosterone, is accounting for their elevated T/E ratios, much as the "nasal inhaler defense" was used by methamphetamine abusers in the 1980s and early 1990s.

It is well established that DHEA is not converted to testosterone in muscle, and any increased production of testosterone in target tissues does not appear to "leak" into the circulation (10,11). The steroids produced in peripheral tissues are used locally, and only inactive metabolites are
released into the circulation. It is possible, however, that testosterone produced locally, used on site, metabolized, and excreted as the common glucuronide conjugate, could contribute to urine testosterone levels, thereby affecting the individual's urinary T/E ratio. Although serum levels of free testosterone are not elevated as a result of DHEA administration (10), urine levels could increase. This may result as a consequence of a high first-pass effect contributing to urinary testosterone metabolites and not to systemically active testosterone in the plasma.

If this hypothesis is correct, then it may be possible for peripheral tissues to produce enough testosterone and excrete the corresponding metabolite to significantly alter the T/E ratio in those individuals using/abusing DHEA. The results of this study suggest that it is possible for DHEA to increase urine testosterone levels but that this increase is not sufficiently pronounced to be mistaken for testosterone abuse. Additionally, a single, acute high-dose DHEA administration had no significant effect on the T/E ratio. A study of the effect of chronic, high-dose DHEA consumption is currently underway.

References


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