tion in regard to disease specificity. However, p185 has been detected in cancers of different organs (4, 12, 13), and diagnostic specificity for breast cancer is unlikely. Application of lectin assays to cancer-associated proteins could be an important contribution to distinguishing breast cancer from benign breast lumps. Thus, in this small sample of subjects, a cutoff point was observed below which all patients were negative; 92% of the patients above this value were positive for cancer.

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References

Oral Dehydroepiandrosterone Supplementation Can Increase the Testosterone/Epitestosterone Ratio, Larry D. Bowers (Athletic Drug Testing and Toxicology Laboratory, Indiana University, Indianapolis, IN 46202-5120; fax 317-274-3223, e-mail lbowers@iupui.edu)

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 (Plania punctulata, 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, 11β-hydroxyandrosterone, 11β-hydroxyetiocholanolone, androst-5-en-3,17-diol, 5a-androst-3a,17β-diol, 5β-androst-3a,17β-diol, dihydrotestosterone, DHEA, T, and E were purchased from either Sigma Chemical Co. or Steraloids and were used as received. The d3-testosterone and d4-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 d3-testosterone, 15 μg/L d4-epitestosterone, and 50 μg/L of methyltestosterone were added as internal standards, was loaded onto a preconditioned C18 solid-phase extraction cartridge, washed with water, and eluted with methanol. The samples were hydrolyzed at pH 7 with β-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, 11β-hydroxy-androsterone, 11β-hydroxy-etiocholanolone, 5α-androsten-3,17-diol, 5α-androstan-3α,17β-diol, and 5β-androstan-3α,17β-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 given to subjects.

Table 1. Steroid ratios and concentrations in the urine of subjects after 3 days of ingestion of DHEA.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age, years</th>
<th>Product</th>
<th>Dose, mg/L</th>
<th>T/E ratio</th>
<th>Testosterone μg/L</th>
<th>Testosterone μg/g creat</th>
<th>Epistosterone μg/L</th>
<th>Epistosterone μg/g creat</th>
<th>Andro μg/g creat</th>
<th>Etio μg/g creat</th>
<th>DHT μg/g creat</th>
<th>5β/5α-Diol ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47</td>
<td>B</td>
<td>Pre-dose</td>
<td>2.4</td>
<td>6</td>
<td>14</td>
<td>2.5</td>
<td>6</td>
<td>1600</td>
<td>930</td>
<td>85</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>50</td>
<td>8.1</td>
<td>22</td>
<td>55</td>
<td>2.7</td>
<td>6.8</td>
<td>6600</td>
<td>2000</td>
<td>8.1</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>100</td>
<td>11.4</td>
<td>39</td>
<td>85</td>
<td>3.4</td>
<td>7.4</td>
<td>7900</td>
<td>4200</td>
<td>21</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>150</td>
<td>14.4</td>
<td>90</td>
<td>112</td>
<td>6.3</td>
<td>6.2</td>
<td>22000</td>
<td>18000</td>
<td>88</td>
<td>63</td>
</tr>
<tr>
<td>2</td>
<td>61</td>
<td>B</td>
<td>Pre-dose</td>
<td>1.3</td>
<td>31</td>
<td>26</td>
<td>27</td>
<td>22</td>
<td>2600</td>
<td>2000</td>
<td>10</td>
<td>3.7</td>
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<td></td>
<td></td>
<td>A</td>
<td>50</td>
<td>1.6</td>
<td>67</td>
<td>38</td>
<td>47</td>
<td>26</td>
<td>9300</td>
<td>11000</td>
<td>20</td>
<td>7.5</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>B</td>
<td>Pre-dose</td>
<td>1.7</td>
<td>44</td>
<td>33</td>
<td>26</td>
<td>20</td>
<td>1700</td>
<td>1200</td>
<td>6</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>100</td>
<td>3.9</td>
<td>131</td>
<td>65</td>
<td>43</td>
<td>23</td>
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<td>19000</td>
<td>32</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>A</td>
<td>Pre-dose</td>
<td>0.8</td>
<td>34</td>
<td>29</td>
<td>32</td>
<td>34</td>
<td>NA</td>
<td>NA</td>
<td>10</td>
<td>1.2</td>
</tr>
</tbody>
</table>

*Creat, creatinine; Andro, androsterone; Etio, etiocholanolone; DHT, 5α-dihydrotestosterone; 5β/5α-Diol ratio, ratio of concentration of 5β-androstan-3α,17β-diol to 5α-androstan-3α,17β-diol.

Subject 4: 5. NA, not applicable. The concentrations of these compounds were so large that even a 100-fold dilution overloaded the column and gave poor accuracy. I estimate that the concentrations were in excess of 100 mg/L.

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/5β 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-3β,17β-diol, androst-5-en-3β,17α-diol, androst-5-en-3β,7α-diol-17-one, and androst-5-en-3β,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 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.

References

Highly Sensitive and Specific Fluorescence Reverse Transcription-PCR Assay for the Pseudogene-free Detection of β-Actin Transcripts as Quantitative Reference, Karl-Anton Kreuzer,1 Ulrich Lass,1 Olbert Landt,2 Andreas Nitsche,1 Jutta Laser,1 Heinz Ellerbrok,3 Georg Pauli,3 Dieter Huhn,1 and Christian Andreas Schmidt1* (1 Department of Medicine, Division of Hematology/Oncology, Charité-Virchow-Klinikum, Humboldt University Berlin, Augustenburger Platz 1, 13353 Berlin, Germany; 2 TIB Molbiol Inc., 10829 Berlin, Germany; 3 Robert Koch Institute, Department of Virology, 13353 Berlin, Germany; * author for correspondence: fax 49-30-450-53395, e-mail cschmidt@charite.de)

The use of common reverse transcription (RT)-PCR reference target sequences can produce false-positive results by amplification of either contaminating DNA or processed pseudogenes. Furthermore, qualitative RT-PCR alone cannot distinguish between high- and poor-quality cDNA preparations, which again may be crucial for the interpretation of low-abundance transcripts. We have developed a highly sensitive quantitative RT-PCR for β-actin, using the TaqMan™ chemistry. Through this technique, we were able to quantitatively detect 10 β-actin molecules per 100 ng of cDNA without coamplification of pseudogenes or genomic DNA. Thus, the presented method may be advantageous for the interpretation of quantitative RT-PCR results.

PCR analysis may be difficult to interpret unless a reference target sequence is amplified in parallel. This control reaction is necessary to evaluate whether a sufficient amount of amplifiable material is present in the sample investigated. Thus, negative PCR can be defined as such only when amplification of a reference gene reveals a positive result. Amplifying a reference is especially important in RT-PCR because RNA can be degraded rapidly before or during cDNA synthesis.

As reference sequences for RT-PCR, so called “housekeeping” genes are preferred because they are constitutionally expressed by all cell types. β-Actin is an attractive candidate for reference coamplification because it exhibits only minor intra-individual kinetic changes and is not primarily affected by any human disease (1). However, a major concern with β-actin and other commonly used references such as glyceraldehyde-3-phosphate dehydrogenase is that processed pseudogenes can be coamplified