Trace Contamination of Over-the-Counter Androstenedione and Positive Urine Test Results for a Nandrolone Metabolite

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Context Several anabolic steroids are sold over-the-counter (OTC) in the United States, and their production is not regulated by the US Food and Drug Administration. Reports have suggested that use of these supplements can cause positive urine test results for metabolites of the prohibited steroid nandrolone.

Objectives To assess the content and purity of OTC androstenedione and to determine if androstenedione and 19-norandrostenedione administration causes positive urine test results for 19-norandrosterone, a nandrolone metabolite.

Design Randomized controlled trial of androstenedione, open-label trial of 19-norandrostenedione, and mass spectrometry of androstenedione preparations, conducted between October 1998 and April 2000.

Setting Outpatient facility of a university hospital.

Participants A total of 41 healthy men aged 20 to 44 years.

Intervention Participants were randomly assigned to receive oral androstenedione, 100 mg/d (n=13) or 300 mg/d (n=11) for 7 days, or no androstenedione (n=13); in addition, 4 patients received 10 µg of 19-norandrostenedione.

Main Outcome Measures Content of OTC androstenedione preparations; level of 19-norandrosterone in urine samples, determined by mass spectrometry, compared among the 3 randomized groups at day 1 and day 7, and among the participants who received 19-norandrostenedione from October 1998 to April 2000.

Results All urine samples from participants treated with androstenedione contained 19-norandrosterone, while no samples from the no-androstenedione group did. Urinary concentrations were averaged for day 1 vs day 7 measurements; mean (SD) 19-norandrosterone concentrations in the 100-mg/d and 300-mg/d groups were 3.8 (2.5) ng/mL and 10.2 (6.9) ng/mL, respectively (P=.006). The 19-norandrosterone content exceeded the cutoff for reporting positive cases (>2.0 ng/mL) in 20 of 24. The androstenedione preparation used was pure at a sensitivity of 0.1%, but at 0.001% 19-norandrostenedione was found. For the 4 participants to whom 10 µg of 19-norandrostenedione was administered, 19-norandrosterone was found in all urine samples. Of 7 brands of androstenedione analyzed at the 1% level, 1 contained no androstenedione, 1 contained 10 mg of testosterone, and 4 more contained 90% or less of the amount stated on the label.

Conclusion Our study suggests that trace contamination of androstenedione with 19-norandrostenedione is sufficient to cause urine test results positive for 19-norandrosterone, the standard marker for nandrolone use. Oral steroid doses as small as 10 µg are absorbed and excreted in urine. Some brands of androstenedione are grossly mislabeled. Careful analysis of androstenedione preparations is recommended in all studies of its biological effects.

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lone itself is rarely found in urine, these cases are actually reports of findings of urinary 19-norandrosterone, the major but inactive metabolite of nandrolone (19-nortestosterone). Administration of nandrolone fell out of style in the 1980s because athletes know that it is readily detectable in urine for weeks.3 Our laboratory conducts approximately 15,000 tests per year and reported 10, 23, and 35 cases where 19-norandrosterone was found in urine in 1997 to 1999, respectively.

19-Norandrosterone is also found in urine after ingestion of the OTC steroid 19-norandrostenedione.8 In sport, the origin of urinary 19-norandrosterone is moot because many sports organizations prohibit and test for OTC steroids in addition to controlled steroids. 19-Norandrosterone traces may occur naturally in the urine of untreated males7,8; norandrosterone excretion and examination of impurities at more than 0.1% of androstenedione. Both brands were more than 99.9% pure, and brand 1 was selected (TABLE 1).

Initial Androstenedione Capsule Analysis
We analyzed 18 capsules from brands 1 and 2 to select a brand for the androstenedione clinical study. This analysis detected impurities at greater than 0.1% of androstenedione. The mean androstenedione content of the brands labeled as containing 100 mg varied from 85 to 103 mg. The amounts of androstenedione in brands 1, 3, and 4 were within ±10% of that stated on the label, whereas brands 2 and 5 through 9 contained less than 90%.

Androstenedione Administration Study
19-Norandrosterone was found in the urine samples of all subjects (n=24; range, 1.3–23.1 ng/mL) who received androstenedione, in none of the baseline urine samples (n=37), and in none of the day 1 (n=13) or day 7 urine samples (n=13) from the 0-mg/d group (TABLE 2). There was no difference in 19-norandrosterone concentrations between day 1 and day 7 (P=.06 and P=.67 for the 100 mg/d and 300 mg/d groups, respectively); thus, the mean concentrations of day 1 and day 7 were averaged. The mean 19-norandrosterone concentrations in the 100- and 300-mg/d groups were 3.8 (2.5) ng/mL and 10.2 (6.9) ng/mL, respectively. These concentrations differed (P=.006), indicating dose dependency (TABLE 2).
Reanalysis of Brand 1
After finding significant amounts of 19-norandrosterone in the urine samples from men who received brand 1, 2 capsules from each of 4 bottles of brand 1 androstenedione were reanalyzed using a method 100 times more sensitive to detect 19-norsteroids if present at a level greater than 0.001% of the androstenedione. None of the capsules contained nandrolone or 19-norandrostenol; however, 7 of 8 contained 0.004% to 0.018% 19-norandrostenedione (mean, 0.0076%) or 7.6 µg per capsule.

19-Norandrostenedione Administration Study
The urine samples of all 4 subjects who ingested 10 µg of 19-norandrostenedione contained both 19-norandrosterone and 19-noretiocholanolone (Table 3), neither of which was found in the baseline urine samples. The 8-hour recovery of 19-norandrostenedione, measured as equivalents of 19-norandrosterone and 19-noretiocholanolone, was 48% (range, 32%-66%) of the total 10-µg dose.

TABLE 1. Summary of the Purity and Content of Capsules of Over-the-Counter Androstenediones

<table>
<thead>
<tr>
<th>Brand No.</th>
<th>No. of Capsules Tested</th>
<th>Steroid and Dose Listed on Label†</th>
<th>Steroids Found</th>
<th>Mean Amount, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1‡</td>
<td>13</td>
<td>Androstenedione, 100 mg</td>
<td>Androstenedione</td>
<td>93.1§</td>
</tr>
<tr>
<td>2‡</td>
<td>5</td>
<td>Androstenedione, 100 mg</td>
<td>Androstenedione</td>
<td>82.8§</td>
</tr>
<tr>
<td>3‡</td>
<td>4</td>
<td>Androstenedione, 100 mg</td>
<td>Androstenedione</td>
<td>103</td>
</tr>
<tr>
<td>4‡</td>
<td>2</td>
<td>Androstenedione, 100 mg</td>
<td>Androstenedione</td>
<td>90</td>
</tr>
<tr>
<td>5‡</td>
<td>4</td>
<td>Androstenedione, 100 mg</td>
<td>Androstenedione</td>
<td>88</td>
</tr>
<tr>
<td>6‡</td>
<td>4</td>
<td>Androstenedione, 100 mg</td>
<td>Androstenedione</td>
<td>85</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>4-Androstene 3, 17-dione, 50 mg¶</td>
<td>4-Androstene</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3, 17-dione</td>
<td>3, 17-dione</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>Androstenedione, 50 mg</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>4-Androstene 3, 17-dione, 250 mg¶</td>
<td>4-Androstene</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3, 17-dione</td>
<td>3, 17-dione</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(not listed on label)</td>
<td>Testosterone</td>
<td>10</td>
</tr>
</tbody>
</table>

*Brands 1 and 2 and 3 through 9 tested for impurities at 0.1% and 1%, respectively.
†Exactly as listed on the label.
‡Brand used for our study in reference 9.
¶Range, 84-107 mg.
§Range, 76-84 mg.
¶4-Androstene 3, 17-dione = androstenedione.

TABLE 2. Number of Subjects Categorized by Concentration and Mean Concentration of 19-Norandrosterone in Urine Samples Collected 0 to 8 Hours After Administration of 100 or 300 mg of Androstenedione

<table>
<thead>
<tr>
<th>Dose, mg</th>
<th>&lt;0.5 ng/mL</th>
<th>0.5-2.0 ng/mL</th>
<th>2.1-5.0 ng/mL</th>
<th>5.1-10 ng/mL</th>
<th>11-35 ng/mL</th>
<th>Mean (SD) Concentration, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.0 (0.0)†</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>3</td>
<td>7</td>
<td>3</td>
<td>0</td>
<td>3.8 (2.5)†</td>
</tr>
<tr>
<td>300</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>4</td>
<td>10.2 (6.0)</td>
</tr>
</tbody>
</table>

*Sports test results were considered negative when concentrations were less than or equal to the 2.0 ng/mL cutoff and positive when concentrations were greater than 2.0 ng/mL.
†Significantly different from the 300-mg dose (P = .008).

TABLE 3. Mean Concentrations of 19-Norandrosterone and 19-Noretiocholanolone After Oral Administration of 10 µg of 19-Norandrostenedione to 4 Subjects

<table>
<thead>
<tr>
<th>Time, h</th>
<th>19-Norandrosterone Mean (Range), ng/mL</th>
<th>19-Noretiocholanolone Mean (Range), ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>17.2 (2.8-48.9)</td>
<td>5.2 (1.1-16.7)</td>
</tr>
<tr>
<td>3-4</td>
<td>5.6 (4.4-7.8)</td>
<td>1.3 (0.6-1.9)</td>
</tr>
<tr>
<td>5-6</td>
<td>1.2 (0.6-1.7)</td>
<td>0.3 (0.1-0.3)</td>
</tr>
<tr>
<td>7-8</td>
<td>0.5 (0.1-0.8)</td>
<td>0.2 (0.1-0.3)</td>
</tr>
</tbody>
</table>
as androstenedione are readily converted to estrogens in humans, and this conversion is associated with C-19 desmethylated to 19-norsteroids.\textsuperscript{11}

We cannot exclude the possibility of stimulation of a latent pathway either. There are reports of up to 0.6 ng/mL of 19-norandrosterone in the urine of untreated males,\textsuperscript{7,8} of 19-nortestosterone and 19-norandrostenedione in human ovarian follicles,\textsuperscript{12} and of 19-nortestosterone in the serum of pregnant women.\textsuperscript{13} However, none of the 37 subjects who received androstenedione or the 4 subjects who received 19-norandrostenedione had 19-norandrosterone levels greater than 0.5 ng/mL in their baseline urine samples. These 41 cases together with the existing 47 cases\textsuperscript{7,8,14} add support for the suggestion that the upper limit of endogenous 19-norandrosterone production does not result in urine concentrations of 19-norandrosterone greater than 0.6 ng/mL.

The hypothesis that the 19-norandrostenedione is derived from the 19-norandrostenedione contamination is strongly supported by the finding that administration of 19-norandrostenedione produced urinary concentrations of 48.9 ng/mL of 19-norandrosterone and 16.7 ng/mL of 19-noretiocholanolone in the first 2 hours and a 48% recovery of the 19-norandrostenedione in urine as the metabolites 19-norandrostosterone and 19-noretiocholanolone. Therefore, the 19-norandrostenedione found in this study is almost certainly due to ingestion of contaminated androstenedione.

The manufacturer of the particular batch of brand 1 androstenedione that we reanalyzed should not be faulted for a product with a maximum contamination of 0.018%. This level of contamination is far below the level of 0.1% accepted by the Food and Drug Administration for pharmaceuticals.\textsuperscript{15} In contrast, some of the other products contained less drug than was listed on the label and even misrepresented ingredients. According to the Dietary Supplement Health and Education Act,\textsuperscript{2} both brand 8 (no androstenedione) and brand 9 (fails to identify testosterone) are misbranded. If we apply the United States Pharmacopoeia\textsuperscript{16} criterion of ±10% for ethical pharmaceuticals, then brands 2, 5, 6, and 7 are misbranded (wrong strength). Thus, 6 of 9 brands tested were misbranded.

Today most steroids are made from precursors, eg, diosgenin from yams and stigmasterol from soya, by semisynthesis\textsuperscript{17} or bacterial action.\textsuperscript{18} These publications do not provide sufficient detail to determine how the contamination of androstenedione with 19-norandrostenedione might have occurred. It is also possible that the contamination occurred after synthesis, for example, during capsulation or some other packaging or formulation step.

Sport organizations that test for anabolic steroids have banned androstenedione even though it is sold OTC in the United States. The data reported herein show that contaminated androstenedione administration will, like nandrolone and 19-norandrostenedione, be detected as 19-norandrosterone in urine and can produce a positive test result for anabolic steroids in subjects taking OTC dietary supplements.

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REFERENCES