Metabolism of Orally Administered Androstenedione in Young Men

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Androstenedione is a steroid hormone and the major precursor to testosterone. It is available without prescription and taken with the expectation that it will be converted to testosterone endogenously and increase strength and athletic performance. The metabolism of orally administered androstenedione has not been well studied.

We randomly assigned 37 healthy men to receive 0, 100, or 300 mg oral androstenedione in a single daily dose for 7 d. Single 8-h urine collections were performed on the day before the start of the androstenedione administration and on d 1 and 7 to assess excretion rates of free and glucuronide-conjugated testosterone, androsterone, etiocholanolone, and dihydrotestosterone. Serum testosterone glucuronide concentrations were measured by frequent blood sampling over 8 h on d 1 in 16 subjects (5 each in the 0 and 100 mg group and 6 in the 300 mg group).

In the control group, mean (±SE) d 1 and 7 excretion rates for testosterone, androsterone, etiocholanolone, and dihydrotestosterone were 3 ± 1, 215 ± 26, 175 ± 26, and 0.4 ± 0.1 µg/h, respectively. In the 100 mg group, mean d 1 and 7 excretion rates for testosterone, androsterone, etiocholanolone, and dihydrotestosterone were 47 ± 11, 3,836 ± 458, 4,306 ± 458, and 1.6 ± 0.2 µg/h, respectively. In the 300 mg group, mean d 1 and 7 excretion rates for testosterone, androsterone, etiocholanolone, and dihydrotestosterone were 115 ± 39, 8,142 ± 1,362, 10,070 ± 1,999, and 7.7 ± 1.5 µg/h, respectively. Urinary excretion rates of all metabolites were greater in both the 100 and 300 mg groups than in controls (P < 0.0001). Urinary excretion rates of testosterone (P = 0.007), androsterone (P = 0.009), etiocholanolone (P = 0.0005), and dihydrotestosterone (P < 0.0001) were greater in the subjects who received 300 mg androstenedione than in those who received 100 mg. In the treated groups, excretion of free testosterone accounted for less than 0.1% of the total excreted testosterone measured. Serum testosterone glucuronide levels increased significantly during frequent blood sampling in both the 100 and 300 mg groups compared with controls (P = 0.0005 for the 100 mg group; P < 0.0001 for the 300 mg group). The mean net changes in area under the curve for serum testosterone glucuronide were −18 ± 25%, 579 ± 572%, and 1,267 ± 1,675% in the groups receiving 0, 100, and 300 mg/d androstenedione, respectively.

We conclude that the administration of both 100 and 300 mg androstenedione increases the excretion rates of conjugated testosterone, androsterone, etiocholanolone, and dihydrotestosterone and the serum levels of testosterone glucuronide in men. The magnitude of these increases is much greater than the changes observed in serum total testosterone concentrations. These findings demonstrate that orally administered androstenedione is largely metabolized to testosterone glucuronide and other androgen metabolites before release into the general circulation. (J Clin Endocrinol Metab 86: 3654–3658, 2001)

Androstenedione is a steroid hormone and an immediate precursor to testosterone and estrone in the intrinsic synthetic pathways of androgens and estrogens (1–3). In the United States, androstenedione is defined as a dietary supplement by the Dietary Supplement Health and Education Act of 1994 and is thus sold over the counter. The number of individuals using androstenedione is unknown, but the dietary supplement industry in general and the pro-hormone market in particular continue to grow rapidly. Recently, it was reported that the dietary supplement industry in the United States alone generates annual sales of 12 billion dollars (4).

Androstenedione is marketed primarily to athletes as a potential anabolic agent and is also claimed to have beneficial effects on general well-being, libido, and quality of life. None of these assertions has been demonstrated in peer-reviewed studies. There is no standard dose of oral androstenedione, although manufacturers generally recommend a dose of 100–300 mg daily. It is widely believed, however, that many users take much higher doses (5).

Oral administration of 100 mg androstenedione increased serum testosterone levels in a small study of two women (6), but did not increase serum testosterone levels in a study of healthy young men (7). Recently, we reported that orally administered androstenedione at a dose of 300 mg daily (but not 100 mg) increases serum testosterone levels in healthy young men (8). The average increase in serum testosterone levels was modest, although there was considerable variability between subjects (8).

In this study we investigated the metabolism of androstenedione in 37 subjects who received no androstenedione or 100 or 300 mg androstenedione daily for 1 wk. Additionally, to delineate the metabolic pathway of orally administered androstenedione in more detail, we measured serum testosterone glucuronide levels directly in a subset of subjects and compared these changes with changes in unconjugated...
total serum levels and urinary excretion of androstenedione and testosterone metabolites.

Subjects and Methods

The details of the subjects and the protocol have been reported previously (8) and are summarized below.

Study subjects

The original study enrolled 42 men between 20 and 40 yr of age (8). Of these, 37 completed all urine collections and are included in the present report. All subjects denied participation in competitive weight-lifting or bodybuilding. Men with a history of cardiopulmonary disease, malignancy, prostate disease, major psychiatric disease, substance abuse, or use in the previous 6 months of any medication known to affect steroid hormone or binding protein levels were excluded. Subjects were also excluded if they reported prior use of androstenedione supplements or androgenic/anabolic steroids. All subjects were required to have normal serum testosterone and creatinine levels and normal liver function tests. Subjects were recruited through approved postings at Massachusetts General Hospital and affiliated institutions and advertisements in local newspapers. The study was approved by the human research committee at Massachusetts General Hospital, and all study participants gave written informed consent.

Protocol

Subjects were randomly assigned to one of three groups: no androstenedione (group 1; n = 13), 100 mg androstenedione (Sports One, Klein Laboratories, Wallingford, CT) daily for 7 d (group 2; n = 13), or 300 mg of androstenedione daily for 7 d (group 3; n = 11). On each of the 7 treatment days the androstenedione capsules were dispensed by a nurse at the General Clinical Research Center at the same time of day (within a 2-h window). Subjects were instructed to ingest nothing by mouth except water for 1 h after androstenedione administration. Serum androstenedione and testosterone concentrations were measured at 0, 15, 30, 45, 60, 90, 120, 180, 240, 360, and 480 min on d 1 and 7. Serum testosterone glucuronide levels were measured at each of these time points on d 1 in a total of 16 subjects selected at random. Urine samples were collected for 8 h on the day before the first treatment day and on d 1 and 7 during the periods of frequent blood sampling. Excretion rates of testosterone, androsterone, etiocholanolone, and dihydrotestosterone (DHT) glucuronides were computed from the urine samples.

Measurements and supplement analysis

Serum measurements. Serum testosterone (Diagnostic Products, Los Angeles, CA) and androstenedione (Diagnostics Systems Laboratories, Inc., Webster, TX) concentrations were measured by RIA. The intra- and interassay coefficients of variation for the testosterone and androstenedione assays were 5.1% and 3.8%, and 10.1 and 8.7%, respectively. The cross-reactivity of androstenedione in the testosterone assay was 0.5%. There was no cross-reactivity of testosterone glucuronide in either the testosterone or androstenedione assay. All samples for an individual subject were analyzed in the same assay.

Serum testosterone glucuronide was measured as follows. Serum (0.5 ml) was extracted with 1.5 ml ethyl acetate/cyclohexane (2:1) to remove unconjugated testosterone from the sample. A solution was prepared of 70 mg β-glucuronidase (product G-0251, Sigma, St. Louis, MO) in 18 ml 0.1 n sodium acetate buffer, pH 5.0. To the spent serum were added 1.5 ml β-glucuronidase solution, and the tubes were incubated overnight at 45 C. After incubation, a 50-μl aliquot was removed from each tube and assayed for testosterone using a kit from Diagnostic Products. Testosterone glucuronide (nanograms per dl) was calculated as: [testosterone (ng/dl) (4)(1.61) /0.645 = testosterone glucuronide (ng/dl)], where 4 is the dilution, 1.61 corrects for mol wt, and 0.645 is the mean recovery calculated from 10 samples. The intra- and interassay coefficients of variation for this assay were 5.7% and 10.4%, respectively.

Analysis of urinary metabolites. The widely different concentrations of steroids in urine required multiple analyses and diluting the urine samples with steroid-free urine. Deuterated testosterone ([16,16,17\(^{3}\)H]testosterone) and etiocholanolone ([2,2,4,4-\(^{3}\)H]etiocholanolone) were added to each urine at concentrations of 40 and 1000 ng/ml, respectively. Steroids were extracted from 2.5 ml urine or diluted urine using a modification of a previously reported method (9). The procedure includes C18 (Varian Associates, Palo Alto, CA) column chromatography, elution with methanol, enzymatic deconjugation with β-glucuronidase from Escherichia coli, extraction with diethyl ether, solvent evaporation, and desiccation in vacuo. Because urine samples were analyzed after deconjugation with β-glucuronidase, both free and glucuronide-conjugated urinary steroids were measured. Urinary free testosterone was measured after ether extraction for three subjects in each group.

Gas chromatographic-mass spectral (5973 quadrupole, Hewlett-Packard Co., Palo Alto, CA) analysis of the trimethylsilyl derivatives used conditions reported previously (9). Data were acquired in the selected ion-monitoring mode that targeted the molecular ion of each steroid. The concentration of testosterone was calculated from the peak height ratio of testosterone to [16,16,17\(^{3}\)H]testosterone. Androsterone and etiocholanolone concentrations were calculated from the peak area ratio of androstenedione or etiocholanolone to [2,2,4,4-\(^{3}\)H]etiocholanolone. Deuterated DHT was not available, and DHT concentrations were determined from a calibration curve. Before calculating excretion rates, the concentrations of steroids were corrected to a specific gravity (SG) of 1.020 using the formula: [corrected] = [uncorrected] X (1.020 – 0.998)/ (SG – 0.998) (10).

Analysis of the androstenedione supplement

Analysis of the androstenedione capsules has been reported previously (8). The mean amount of androstenedione in the 13 capsules, each of which was purported to contain 100 mg androstenedione, was 99.8 mg (range, 83.9–113.1 mg; coefficient of variation, 8.7%). The mass spectrum of the androstenedione peak was identical to that of the reference standard. Other peaks were present in the chromatograms; however, all were less than 1% of the androstenedione peak.

Statistical analysis

Excretion rates of urinary androgen metabolites were compared after log transformation using a repeated measures analysis of covariance with the baseline value as the covariate. The mean net change in the area under the curve (AUC) of serum testosterone glucuronide concentrations (measured in a subset of patients on d 1) was compared after log transformation using analysis of covariance with the baseline value as a covariate. Log transformation was performed due to unequal variance. To assess whether changes in serum testosterone or androstenedione levels correlated to changes in the excretion rates of the urinary metabolites, partial correlation coefficients were calculated between all variables after correcting for dose and log transformation. Data are expressed as the mean ± SEM. All P values are two-sided, and P < 0.05 was considered statistically significant.

Results

The baseline characteristics of our study subjects and changes in serum androstenedione, testosterone, estrone, and estradiol were reported previously (8). All subjects were between the ages of 20–40 yr and were well matched for baseline serum testosterone, androstenedione, estrone, and estradiol concentrations.

There was no significant difference in urinary excretion rates in any of the urinary metabolites between d 1 and 7. Thus, values from d 1 and 7 were combined. There were dramatic increases in urinary excretion rates of free plus glucuronide-conjugated testosterone, androsterone, DHT, and etiocholanolone in both the 100 and 300 mg groups (P < 0.0001 for all comparisons with controls; Fig. 1). Urinary excretion rates of free plus glucuronide-conjugated testosterone (P = 0.007), androsterone (P = 0.009), etiocholanolone (P = 0.0005), and DHT (P < 0.0001) were greater in the subjects who received 300 mg androstenedione than in those
who received 100 mg. Less than 0.1% of the total testosterone measured in the urine was in the form of free testosterone in subjects receiving either dose of androstenedione. Excretion rates of free testosterone did not differ among groups.

There was a considerable individual variability in the testosterone excretion rates of the subjects. The individual excretion rates for each subject in the 300 mg group during each collection are shown in Table 1. The baseline (preandrostenedione) testosterone excretion rates of the two Asian subjects in the 300 mg group were lower than the excretion rates of the other nine subjects in this group. After androstenedione administration, this difference became much more dramatic (Table 1). The mean testosterone excretion for the two Asian subjects on the treated days was less than 1/10th the mean excretion rate of the group as a whole.

Mean testosterone glucuronide levels during the frequent blood sampling period on d 1 in the 16 subjects in whom it was measured are shown in Fig. 2. In the control group serum testosterone glucuronide levels were stable during frequent blood sampling. The net mean changes in AUC for serum testosterone glucuronide were $-18 \pm 25\%$, $579 \pm 572\%$, and $1267 \pm 1675\%$ in the groups receiving 0, 100, and 300 mg/d androstenedione, respectively ($P = 0.0005$ for the 100 mg group; $P < 0.0001$ for the 300 mg group vs. controls).

Table 2 shows the partial correlation coefficients for the excretion rates of the urinary metabolites and the net changes in serum testosterone and androstenedione AUC for subjects in both treated groups (excluding controls). Significant correlations were found between serum testosterone and serum androstenedione AUC ($r = 0.62; P = 0.0016$). There was no significant correlation between either serum testosterone or androstenedione levels and any of the urinary metabolites measured. Significant correlations were observed among all urinary metabolites except between urinary testosterone and DHT excretion rates. Among the 14 subjects in whom both urinary testosterone glucuronide and serum testosterone glucuronide were measured on d 1, the excretion rate of testosterone glucuronide and the net AUC in serum testosterone glucuronide were highly correlated ($r = 0.883$).

**Discussion**

In this study we found that oral administration of both 100 and 300 mg androstenedione increases the urinary excretion rates of androgen metabolites, including testosterone glucuronide, dramatically. Furthermore, we found that the small increases in total serum testosterone levels previously reported in subjects receiving 300 mg androstenedione are accompanied by massive increases in serum testosterone glucuronide (which is not biologically active). Specifically, whereas serum testosterone AUC increased by approximately 34% in the 300 mg group (8), testosterone glucuronide AUC increased more than 10-fold. Furthermore, whereas several studies have now documented that 100-mg or smaller

**TABLE 1.** Urinary testosterone glucuronide excretion rates (micrograms per h) in subjects receiving 300 mg/day androstenedione

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Ethnicity</th>
<th>Baseline</th>
<th>Day 1</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>African American</td>
<td>5.5</td>
<td>247.0</td>
<td>131.5</td>
</tr>
<tr>
<td>2</td>
<td>Caucasian</td>
<td>3.4</td>
<td>32.9</td>
<td>12.8</td>
</tr>
<tr>
<td>3</td>
<td>Caucasian</td>
<td>1.8</td>
<td>32.0</td>
<td>10.7</td>
</tr>
<tr>
<td>4</td>
<td>Caucasian</td>
<td>10.8</td>
<td>290.9</td>
<td>520.9</td>
</tr>
<tr>
<td>5</td>
<td>Caucasian</td>
<td>4.7</td>
<td>416.2</td>
<td>132.5</td>
</tr>
<tr>
<td>6</td>
<td>Caucasian</td>
<td>2.1</td>
<td>191.5</td>
<td>50.2</td>
</tr>
<tr>
<td>7</td>
<td>Asian</td>
<td>0.2</td>
<td>3.8</td>
<td>0.8</td>
</tr>
<tr>
<td>8</td>
<td>Asian</td>
<td>0.2</td>
<td>2.3</td>
<td>3.1</td>
</tr>
<tr>
<td>9</td>
<td>Caucasian</td>
<td>3.7</td>
<td>77.6</td>
<td>126.3</td>
</tr>
<tr>
<td>10</td>
<td>Caucasian</td>
<td>2.6</td>
<td>97.7</td>
<td>34.5</td>
</tr>
<tr>
<td>11</td>
<td>Caucasian</td>
<td>0.3</td>
<td>48.7</td>
<td>58.9</td>
</tr>
</tbody>
</table>
doses of androstenedione fail to increase serum testosterone levels in men (7, 8, 11, 12), administration of 100 mg androstenedione in this study increased serum testosterone glucuronide levels dramatically.

The metabolism of androgens is characterized by enzymatic conversion to more polar inactive compounds (phase I reactions) and conjugation by glucuronic acid or sulfate (phase II reactions) (13). These reactions lead to products that are readily eliminated by the kidney and are almost exclusively in the conjugated form. The initial rate-limiting step in phase I androstenedione metabolism is the reduction of the C4–C5 double bond to yield 5α- and 5β-androstenedione. This reaction is followed by reduction of the 3-keto group to produce androsterone and etiocholanolone, which are end products of androstenedione metabolism (14). Testosterone, which is converted from androstenedione by 17β-hydroxysteroid dehydrogenase, is metabolized similarly as the C4–C5 double bond is reduced to yield 5α- and 5β-DHT, and the 3-keto group is then reduced to produce 5α- and 5β-androstanediols (14, 15). Thus, among the conjugated urinary metabolites measured in this study, androsterone and etiocholanolone should be considered final end products in the androgen degradation pathway, whereas testosterone and DHT represent intermediates (14–18).

Urinary androsterone, etiocholanolone, testosterone, and 5α-DHT excretion were all markedly increased in our subjects. These results are consistent with a recent study that reported that a single dose of 50 mg androstenedione increased urinary excretion of androsterone, etiocholanolone, and testosterone glucuronide transiently in six men (18). Although androstenedione may be converted to androsterone, etiocholanolone, and DHT glucuronides with or without prior conversion to testosterone, urinary testosterone glucuronide is a specific metabolite of unconjugated testosterone (17). 17β-Hydroxysteroid dehydrogenase is more active in the liver than in any other tissue except placenta (19). Orally administered testosterone undecanoate is avidly sulfoconjugated and glucuronically conjugated in splanchnic tissues (20). Studies of iv, radioactively labeled androstenedione administration have shown that androstenedione can be converted to testosterone glucuronide without entering the peripheral circulation (21). Additionally, in our study urinary testosterone glucuronide excretion and serum testosterone glucuronide concentrations increased dramatically after androstenedione administration, whereas serum unconjugated testosterone levels increased only modestly. Taken together, these data strongly suggest that most orally administered androstenedione undergoes first pass hepatic metabolism, initially to testosterone and then to testosterone glucuronide (or testosterone sulfate) before release into the systemic circulation.

The very low urinary testosterone excretion in the Asian subjects taking 300 mg androstenedione is consistent with previous reports that some Asian men excrete less urinary testosterone conjugates than Caucasian men after testosterone administration (16, 18). This difference may be due to more rapid conversion of testosterone to downstream metabolites such as androsterone and etiocholanolone. Additionally, the overall individual variability in the excretion rates of the urinary metabolites in our subjects is consistent with our previous observation that there is wide individual variation in serum sex hormone levels after androstenedione administration (8). The genetic and physiological mechanisms that may underlie this ethnic and individual variation have not been described.

No significant correlations were observed between changes in serum testosterone or androstenedione levels and changes in the urinary metabolites. This finding may be due to the small sample size and wide individual variability.

The finding that oral androstenedione administration increases urinary androgen metabolites dramatically may have an impact on the ability to detect oral androstenedione use in competitive athletics. Even though anabolic effects of androstenedione have not yet been demonstrated, many amateur and professional sports organizations worldwide have banned androstenedione and related prohormones. Thus, a reliable method to detect androstenedione use is needed.

In conclusion, administration of either 100 or 300 mg androstenedione increases urinary excretion rates of conjugated testosterone, androsterone, etiocholanolone, and DHT. These increases are much greater than the modest changes seen in serum total testosterone levels. Furthermore, androstenedione administration increases serum testosterone glucuronide levels to a much greater extent than total unconjugated testosterone. Taken together, these findings suggest that most orally administered androstenedione is metabolized in the liver to testosterone glucuronide and other androgen metabolites before release into the general circulation. This extensive initial metabolism may limit the potential of orally administered androstenedione to increase serum testosterone levels in men.

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**TABLE 2.** Pearson partial correlation coefficients (r) of urinary metabolite excretion rates and net changes in serum testosterone and androstenedione AUC in noncontrol subjects

<table>
<thead>
<tr>
<th></th>
<th>Serum testosterone</th>
<th>Urinary Androsterone</th>
<th>Urinary DHT</th>
<th>Urinary etiocholanolone</th>
<th>Urinary testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum androstenedione</td>
<td>0.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.14</td>
<td>−0.19</td>
<td>−0.03</td>
<td>0.13</td>
</tr>
<tr>
<td>Serum testosterone</td>
<td>0.13</td>
<td>0.18</td>
<td>0.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.72&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urinary androsterone</td>
<td>0.03</td>
<td>0.03</td>
<td>0.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.27</td>
<td>0.76&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urinary DHT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary etiocholanolone</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

<sup>a</sup> P < 0.01.
<sup>b</sup> P < 0.001.
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