

Urinary Excretion of Steroid Metabolites after Chronic Androstenedione Ingestion

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Urinary steroid excretion after androstenedione intake has been examined after a single dose of 50 mg and single doses of 100 or 300 mg/d for 7 d. We evaluated the effects of 28 d of 100 mg three times a day (t.i.d.) androstenedione intake on urinary steroid excretion. Twenty healthy men, ages 30–39 yr (33.5 ± 0.6), consumed 100 mg androstenedione t.i.d. or placebo for 28 d. Urine samples were analyzed for testosterone, epitestosterone, androsterone, and etiocholanolone via HPLC/tandem mass spectrometry on d 0 and 28. Androstenedione intake increased ($P < 0.05$) urinary testosterone 35.1 ± 10.5 ng/ml vs. 251.6 ± 87.5 ng/ml, epitestosterone 35.3 ± 8.8

ng/ml vs. 99.7 ± 28.7 ng/ml, androsterone $2,102 \pm 383$ ng/ml vs. $15,767 \pm 3,358$ ng/ml, and etiocholanolone $1,698 \pm 409$ ng/ml vs. $11,329 \pm 2,656$ ng/ml (means \pm SE). Although the testosterone to epitestosterone ratio (T/E) tended to increase with androstenedione intake (1.2 ± 0.3 vs. 4.0 ± 1.6 ; $P = 0.12$), only one subject had a urinary T/E greater than the current Olympic criteria (>6.0) for a positive drug test. Chronic intake of 100 mg androstenedione t.i.d. increases the urinary excretion of steroid metabolites. Due to inconsistent increases in the T/E ratio, the T/E ratio may not effectively detect androstenedione use. (*J Clin Endocrinol Metab* 89: 6235–6238, 2004)

THE ANDROGENIC HORMONE androstenedione is marketed as a nutritional supplement to increase serum testosterone concentrations. However, when androstenedione is taken in single doses up to 200 mg (1–6) or taken as 100 mg three times a day (t.i.d.) (2, 7), serum testosterone concentrations are not changed in young men (<30 yr). In contrast, when androstenedione is taken as 100 mg t.i.d. by men over 30 yr (8, 9) or in single doses of 300 mg (3), serum testosterone concentrations are slightly elevated.

Due to its structural similarity to testosterone and its purported testosterone-enhancing potential, androstenedione use is prohibited in many sports. Olympic regulations are that a urinary testosterone to epitestosterone (T/E) ratio above 6.0 indicates use of prohibited steroids (9). However, the effects of androstenedione intake on the T/E ratio are not clear.

Uralets and Gillette (10) reported that ingesting a single dose of 50 mg androstenedione results in a T/E ratio above 6.0 for approximately 5 h after intake. Conversely, Catlin *et al.* (11) reported that the T/E ratio is increased to only approximately 4.0 during the 8 h after ingesting a single dose of 100 or 300 mg androstenedione. The effects of taking androstenedione several times per day for prolonged times are unknown but are of keen interest because athletes likely use androstenedione in this manner. In addition, there is evidence that the serum hormonal response to androstenedione intake is reduced with prolonged androstenedione intake (2, 7, 12, 13), which suggests that urinary steroid clearance may be increased with prolonged androstenedione intake. The purpose of this project was to evaluate the effect of ingesting 100 mg androstenedione t.i.d. for 28 d on urinary steroid excretion.

Abbreviations: DHT, Dihydrotestosterone; PL, placebo; T/E, testosterone to epitestosterone ratio; t.i.d., three times a day.

JCEM is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.

Subjects and Methods

Subjects

Twenty men, aged 30–39 yr (Table 1), were recruited from the university and local community to participate in this project, which was approved by the Human Subjects Committee at Iowa State University. All subjects signed an informed consent and completed a written medical history to eliminate any subjects with a known chronic disease. Before participating in this study, subjects were questioned to ensure they were not currently or previously using nutritional supplements. The subjects used in this project were a subset from a larger project, and the serum hormonal and anthropometric data for these subjects have been presented elsewhere (8, 9). Nineteen of the subjects were Caucasian and one subject in the androstenedione group was Asian.

Supplementation

Subjects were randomly assigned in a double-blind manner to consume unmarked white capsules containing either 300 mg/d androstenedione taken in doses of 100 mg t.i.d. (Experimental and Applied Sciences, Golden, CO), or placebo (PL). An independent laboratory (Integrated Biomolecule, Tucson, AZ) verified purity ($\sim 99\%$) and content of the androstenedione capsules via HPLC. No other steroids were present in the supplements. Subjects were instructed to consume the capsules daily before 0900 h, at 1500 h, and before bedtime throughout the 4-wk treatment period. Compliance was monitored through written records and the return of unused supplements at the completion of the study.

Diet and activity

Throughout the 4 wk of the study, subjects were instructed to maintain their normal diet and activity patterns. Instructions were given verbally and in writing to subjects on the procedures for maintaining a diet, medication, and exercise record. These records were maintained for the 2 d before each sampling period.

Urine sample collection and analysis

On d 0 and again on d 28, 8–10 h after the last dose of androstenedione had been ingested, clean-catch midstream urine samples were collected (30 ml) immediately upon waking from an overnight fast. Samples were preserved with 1 g% of sodium azide and frozen at -80 C until analyzed. Urine samples were analyzed for testosterone glucuronide, epitestos-

terone, androsterone, and etiocholanolone via HPLC/tandem mass spectrometry (HPLC/MS/MS) as described previously (14). Analyses were performed at the Indiana University Athletic Drug Testing and Toxicology Laboratory (Indianapolis, IN).

Blood sample collection and analysis

Fasting blood samples were collected between 0630 and 0800 h once per week on the same day each week. Subjects reclined while blood samples were obtained without stasis from an antecubital vein. Blood samples were immediately placed in an ice bath until centrifugation and serum separation. Serum concentrations of estradiol, total testosterone, free testosterone, and androstenedione were measured with commercial RIA kits (Diagnostic Products Corp., Los Angeles, CA, and Diagnostic Systems Laboratory, Webster, TX). Commercially available ELISAs were used to measure serum concentrations of dihydrotestosterone (DHT; Immuno-Biological Laboratories, Hamburg, Germany). All samples for each subject were analyzed in duplicate within the same assay, and the intraassay coefficients of variation for total testosterone, free testosterone, estradiol, androstenedione, and DHT were 6.1, 7.2, 6.6, 5.8, 3.2, and 6.6%, respectively. According to the suppliers of the RIA and ELISA kits, there is no detectable cross-reactivity of the assays for androstenedione, DHT, estradiol, or testosterone.

Calculations and statistics

Data were analyzed using a two-factor (time and treatment) repeated-measures ANOVA (SPSS, Inc., Chicago, IL). When a significant F ratio ($P < 0.05$) was obtained, a Newman-Keuls *post hoc* comparison was used to locate significant differences. To examine the correlation between changes in serum hormones and urinary steroid metabolites, partial correlation coefficients were calculated between changes from d 0 to 28 in all variables. Data are presented as means \pm SE.

Results

Subjects

Body mass or body mass index did not change during the course of the study. All subjects completed the 28-d trial with high (>99%) compliance to the supplementation schedule.

Dietary intake

There was no difference between treatment groups in the composition of the diet during the 2 d before urine sample collection (Table 2). There was also no difference in dietary composition between the first and last urine sample collection.

TABLE 1. Subject descriptive data

	Androstenedione (n = 10)		PL (n = 10)	
	Before	After	Before	After
Age (yr)	32.9 \pm 0.8		34.0 \pm 1.0	
Height (cm)	179.6 \pm 2.7		181.0 \pm 4.1	
Body mass (kg)	84.1 \pm 4.6	83.6 \pm 4.5	96.0 \pm 6.4	95.5 \pm 6.5
Body mass index (kg/m ²)	26.1 \pm 1.3	25.9 \pm 1.3	29.5 \pm 2.2	29.4 \pm 2.2

Values are expressed as mean \pm SE.

TABLE 2. Daily dietary composition for the 2 d preceding urine sample collection

	Androstenedione (n = 10)		PL (n = 10)	
	Before	After	Before	After
kcal/d	2338 \pm 398	2383 \pm 226	2380 \pm 167	2340 \pm 179
Carbohydrate (g)	364.0 \pm 57.7	331.5 \pm 35.2	330.9 \pm 28.9	340.3 \pm 25.7
Fat (g)	80.7 \pm 13.1	75.7 \pm 11.5	78.1 \pm 8.3	76.3 \pm 12.4
Saturated fat (g)	27.6 \pm 4.7	27.7 \pm 4.9	25.9 \pm 3.5	26.1 \pm 4.9
Cholesterol (mg)	385.3 \pm 79.6	272.4 \pm 72.4	311.3 \pm 59.6	203.6 \pm 38.2

Values are expressed as mean \pm SE.

Serum hormonal changes

Four weeks of androstenedione intake increased serum free testosterone, dihydrotestosterone, androstenedione, and estradiol concentrations ($P < 0.05$) but not serum total testosterone concentrations (Table 3). Although serum total testosterone concentrations were not changed by androstenedione intake, there was a significant ($P < 0.05$) correlation between the changes in serum DHT and serum total testosterone ($r^2 = 0.85$). A more detailed presentation of the serum hormonal response in these subjects can be found elsewhere (9).

Urinary steroid excretion

Urinary testosterone, epitestosterone, androsterone, and etiocholanolone concentrations did not change in PL (Table 4). Ingesting 100 mg androstenedione t.i.d. for 28 d increased ($P < 0.05$) urinary testosterone glucuronide concentrations by 839%. The increase in urinary testosterone glucuronide concentration in the Asian subject was considerably lower (200%). The changes in urinary testosterone glucuronide concentrations were not correlated to changes in any serum or urinary hormones.

Ingesting 100 mg androstenedione t.i.d. for 28 d increased ($P < 0.05$) urinary epitestosterone concentrations by 256% in the Caucasian subjects and 438% in the Asian subject. The changes in urinary epitestosterone concentrations were not correlated to changes in any serum or urinary hormones.

Ingesting 100 mg androstenedione t.i.d. for 28 d increased ($P < 0.05$) urinary androsterone concentrations by 925 and 3469% in the Asian subject. The changes in urinary androsterone concentrations were correlated ($P < 0.05$) to the changes in serum DHT ($r^2 = 0.85$) and free testosterone ($r^2 = 0.92$).

Ingesting 100 mg androstenedione t.i.d. for 28 d increased ($P < 0.05$) urinary etiocholanolone concentrations by 567% and did not change appreciably in the Asian subject (+6%). The changes in urinary etiocholanolone concentrations were correlated ($P < 0.05$) to changes in serum androstenedione ($r^2 = 0.90$) and free testosterone ($r^2 = 0.92$).

The T/E ratio was not increased from d 0 to d 28 ($P = 0.12$) by androstenedione intake but decreased 62% in the Asian

TABLE 3. Serum hormone concentrations before and after 4 wk of androstenedione supplementation

	Androstenedione (n = 10)		Placebo (n = 10)	
	Before	After	Before	After
Serum androstenedione (ng/ml)	2.7 ± 0.4	8.4 ± 1.6 ^a	2.6 ± 0.2	2.9 ± 0.2
Serum free testosterone (ng/ml)	21.9 ± 2.9	28.1 ± 2.8 ^a	20.6 ± 1.5	21.6 ± 1.7
Serum total testosterone (ng/ml)	592.2 ± 69.7	633.7 ± 92.9	565.0 ± 27.0	614.4 ± 26.7
Serum DHT (pg/ml)	633.8 ± 43.3	959.1 ± 77.0 ^a	585.3 ± 68.1	579.8 ± 58.2
Serum estradiol (pg/ml)	51.1 ± 8.4	82.0 ± 11.3 ^a	45.8 ± 6.3	48.5 ± 5.8

Values are expressed as mean ± SE.

To convert androstenedione from ng/ml to nmol/liter, multiply by 3.45. To convert free testosterone from pg/ml to pmol/liter, multiply by 3.47. To convert total testosterone from ng/ml to nmol/liter, multiply by 0.0347. To convert DHT from pg/ml to pmol/liter, multiply by 3.44. To convert estradiol from pg/ml to nmol/liter, multiply by 0.0037.

^a Week 4 significantly different from wk 0 ($P < 0.05$) for androstenedione.

TABLE 4. Urinary steroid metabolite excretion before and after 4 wk of androstenedione supplementation

	Androstenedione (n = 10)		PL (n = 10)	
	Before	After	Before	After
Testosterone glucuronide (ng/ml)	26.8 ± 7.1	251.6 ± 92.2 ^a	43.5 ± 11.8	38.0 ± 9.6
Epitestosterone (ng/ml)	28.0 ± 6.0	99.7 ± 30.2 ^a	56.3 ± 13.1	32.6 ± 3.9
Androsterone (ng/ml)	1537 ± 305	15,767 ± 4335 ^a	2620 ± 476	2522 ± 428
Etiocolanolone (ng/ml)	1698 ± 565	11,329 ± 3428 ^a	2211 ± 611	1567 ± 324
Testosterone: epitestosterone	1.2 ± 0.4	4.0 ± 1.8	1.2 ± 0.3	1.5 ± 0.4

Values are expressed as mean ± SE. To convert testosterone and epitestosterone from ng/ml to nmol/liter, multiply by 3.47. To convert androsterone and etiocolanolone from ng/ml to nmol/liter, multiply by 3.49.

^a Week 4 significantly different from wk 0 ($P < 0.05$) for androstenedione.

subject. One Caucasian subject exhibited an extraordinarily large increase in the urinary T/E ratio (from 2 to 17; Fig. 1). Because removal of the data for this subject did not change the overall interpretation of the data set, values for this subject are included throughout the text.

Discussion

The principal finding of this study was that chronic intake of androstenedione markedly increases the urinary excretion of steroid metabolites, including testosterone glucuronide. However, when measured approximately 8–10 h after the last dose of androstenedione, the urinary T/E ratio is not uniformly elevated above the level necessary for disqualification from Olympic athletic competition, even though serum free testosterone concentrations were elevated approximately 40%.

Despite large increases in serum androstenedione concentrations after androstenedione intake, it has been repeatedly demonstrated that ingesting 100 mg androstenedione does not elevate serum total testosterone concentrations in men (2–9, 15). The lack of change in serum testosterone is in contrast to the very large increases in urinary excretion of biologically inactive testosterone glucuronide demonstrated here and elsewhere (4, 10). Taken together, these findings are in agreement with those of Horton and Tait (16), who observed that the vast majority of ingested androstenedione is catabolized into inactive substances in the liver rather than converted into biologically active compounds in the peripheral tissues.

There appears to be a reduction in the serum hormonal response to androstenedione intake with prolonged use (2, 7, 12, 13), indicating that prolonged androstenedione intake may result in either enhanced clearance or reduced absorption of the ingested androgen. Leder *et al.* (4) observed that the urinary excretion rate of steroid metabolites was not

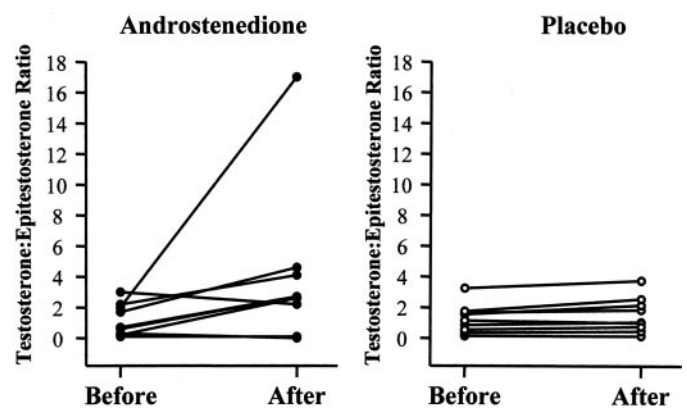


FIG. 1. Urinary T/E ratio for each subject before and after ingesting PL or 100 mg androstenedione t.i.d. for 28 d.

different between d 1 and 7 of androstenedione intake. The current data, along with those after a single dose of 50 mg androstenedione (10) or a single dose of 100 or 300 mg androstenedione taken for 7 d (4), suggest there is no difference in acute *vs.* chronic excretion of steroid metabolites. However, our previous observations (2, 7) of a reduced serum hormonal response to androstenedione intake between 5 and 8 wk of androstenedione intake suggests that changes in absorption or excretion of ingested androstenedione may take longer than 4 wk to occur.

There is a large individual variability in both serum and urinary responses to androstenedione intake (3, 4, 9–11). This variability in the response to androstenedione is highlighted by our observation of one Caucasian subject with a urinary T/E ratio of 17, whereas the rest were less than 3. Despite the individual variability in serum and urinary responses to androstenedione intake, our present results and those of others (4, 11, 16) indicate that the primary fate of ingested

androstenedione is conversion into inactive compounds such as androsterone and etiocholanolone. The cause for the intersubject variability in the serum and urinary hormonal response to androstenedione intake is unclear, although ethnic background (4, 10) and age (8, 9, 15) appear to influence the response to ingested androgens.

In the present study, the Asian male had an approximately 4-fold greater increase in androsterone and an approximately 2-fold greater increase in epitestosterone excretion than did the Caucasian subjects. Additionally, the increased urinary testosterone excretion in the Asian male was approximately one fourth of that observed in the Caucasian males. It has previously been observed that Asian men have a much lower excretion of testosterone glucuronide than Caucasian men after androstenedione intake (4, 10). Leder *et al.* (4) postulated that Asian men experience a much greater conversion of ingested androstenedione to androsterone. Additionally, the present results suggest that Asians may have a lower conversion of androstenedione to testosterone than Caucasians as demonstrated by the greater epitestosterone excretion in the Asian subject. Despite the small numbers of subjects included in this report, the present results are consistent with earlier reports that there are ethnic differences in the metabolism of ingested androstenedione. In addition to ethnicity, there are undoubtedly other, currently unknown, factors that influence the hepatic clearance and metabolism of ingested androgens.

Androstenedione is classified as an anabolic agent and banned in many sports. Androstenedione intake in doses up to 100 mg t.i.d. does not enhance the adaptations to resistance training in men apparently due to a failure to increase serum testosterone concentrations (2, 7, 13, 17). A single dose of 300 mg androstenedione may cause a modest (34%) and transient increase in serum testosterone concentrations (3). Although the effects of larger doses of androstenedione on muscle size and strength have not been studied, anabolic effects are observed only when testosterone is increased to a much greater extent for a more prolonged period (*i.e.* chronic elevation by more than 450%) (18). Even though androstenedione use has not been shown to increase muscle mass or strength, it is widely used (19, 20), so effective detection is necessary to deter its use. It will be difficult to detect and/or deter androstenedione in those sports in which androstenedione use is forbidden because the commonly used T/E ratio does not effectively detect androstenedione use 8–10 h after the last dose. Therefore, adoption of an effective technique for detecting androstenedione use, such as the carbon mass ratio or 6 α -hydroxyandrostenedione (11), is warranted.

In conclusion, when measured approximately 8–10 h after intake, 100 mg androstenedione t.i.d. increases the urinary excretion of steroid metabolites but does not uniformly increase the urinary T/E ratio above the Olympic threshold for disqualification. Therefore, a detection method other than the T/E ratio should be used.

Acknowledgments

Received October 6, 2003. Accepted March 9, 2004.

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This work was supported by Experimental and Applied Science (Golden, CO).

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