Effect of Androstenedione Ingestion on Plasma Testosterone in Young Women; a Dietary Supplement with Potential Health Risks, Andrew T. Kicman, Thomas Bassindale, David A. Cowan, Sian Dale, Andrew J. Hutt, and Anthony R. Leeds (1 Drug Control Centre and Department of Pharmacy, and 2 Department of Nutrition, King’s College London, Franklin-Wilkins Building, 150 Stamford St., London SE1 9NN, United Kingdom; * author for correspondence: fax 44-020-7848-4980, e-mail andrew.kicman@kcl.ac.uk)

Androstenedione is readily available as a “dietary supplement” and can be sold without restriction in most countries, including the US and UK. Manufacturers market androstenedione as a “prohormone” that can increase blood testosterone concentration, thereby increasing lean body mass and strength. It is available in capsules purported to contain 50, 100, or 300 mg of the steroid, and the manufacturers suggest that the “desired” effects can be gained by taking 100–300 mg/day orally. However, a single dose of 100 mg/day is insufficient to increase serum testosterone concentrations in men (1–4). Even with higher “recommended” doses of either 200 or 300 mg/day (given as either single or multiple administrations), no significant increase has been observed in most studies (1, 3, 5), albeit in one investigation, 4 of 14 men had testosterone concentrations that exceeded the reference interval (2). The contribution of these administered amounts of androstenedione to the plasma testosterone concentration appears to be relatively small compared with the large amount of testosterone secreted by the testes.

No comprehensive study has been reported regarding the effect of oral androstenedione administration on plasma testosterone in women. However, there is an indication that it can be increased considerably, based on limited data from two women, obtained by a methodology based on paper chromatography and conversion of androgens to estrogens for fluorometric analysis (6). The plasma concentration of testosterone in women of a reproductive age is ~1/10th that found in men, and the proportion arising from peripheral conversion is much greater (7). Although ~14% of androstenedione in blood is converted peripherally to testosterone in both men and women, the amount converted accounts for approximately one-half the circulating testosterone in the female (7, 8). Hence, ingestion of even a single 100 mg capsule of androstenedione is likely to increase circulating testosterone in women. To test this premise and to examine the extent of increase, we administered 100 mg of androstenedione or placebo in a double-blind crossover study.

Androstenedione (Sigma-Aldrich) was found to contain 9 mg/g testosterone, as quantified, after silylation (9, 10), by gas chromatography–mass spectrometry operated in the selected-ion mode with use of a temperature program (11) and an extraction procedure (12) described elsewhere. To counter speculation that changes in plasma testosterone after androstenedione administration could be confounded by the presence of small amounts of this contaminant, we purified the androstenedione by flash chromatography (13), using a column of alumina (Brockman Grade 1, neutral; Sigma-Aldrich) with cyclohexane–ethyl acetate (1:1 by volume) as the mobile phase. Portions of the eluent fractions were spotted on alumina thin-layer chromatography plates, along with androstenedione and testosterone calibrators, and run with the same mobile phase mixture as for the column. Spots were visualized under ultraviolet light (254 nm), and eluent fractions corresponding to thin-layer chromatography lanes showing the sole presence of androstenedione were pooled and dried. The steroid was then recrystallized from hexane–ethyl acetate (9:1 by volume). Analysis by gas chromatography–mass spectrometry showed no detectable testosterone (limit of detection <<1 mg/g). In addition, full-scan mass spectrometric data showed only characteristic ions for the bis-trimethylsilyl derivative of androstenedione (m/z 430, 415, and 234); no other chromatographic peak >0.1% of the androstenedione signal was observed. The purified androstenedione was ground and mixed (triturated) with lactose (1:3, by weight) with use of a pestle and mortar; hard gelatin capsules were then filled accurately with 400 mg of the mixture (containing 100 mg of androstenedione per capsule). Placebo capsules were prepared containing 400 mg of lactose only.

Ten healthy female volunteers (20–32 years of age) each received a capsule containing either placebo or steroid with a 2-week washout period between administrations. To avoid the possibility of androstenedione being administered to a pregnant woman, the effects of exposure being unknown on the fetus, an inclusion criterion was that participants were taking oral contraceptives. An exclusion criterion was a positive urine test for human chorionic gonadotropin 24 h before each administration. Our local research ethics committee for human studies approved the study, and the volunteers gave written, informed consent.

Blood was collected via a cannula inserted into an arm vein; samples were collected immediately before administration (0 h), every 15 min initially up to 1.5 h after administration, then at 2, 2.5, 3, 4, 5, 6, and 8 h. A final sample was collected by venipuncture at 24 h. The plasma was analyzed for total testosterone (free plus protein-bound) and androstenedione by commercial RIA (Coat-A-Count; DPC®, the testosterone reference interval was quoted as being from nondetectable to 2.8 nmol/L for ovulating women. The cross-reactions of the testosterone assay were determined at 50% displacement of the activity measured in the zero calibrant (50% B/Bo) and were 2.8% for 5a-dihydrotestosterone (a metabolite of testosterone) but negligible for testosterone glucuronide (0.01%). Cross-reactivity with androstenedione was 0.16%, which was not sufficient to interfere with the testosterone assay after androstenedione treatment. Between-assay CVs (n = 10) were 9.5%, 8.5%, and 7.3% at plasma testosterone concentrations of 5.4, 12.6, and 35.4 nmol/L, respectively. The androstenedione assay was validated. Between-
assay CVs (n = 12) were 9.6% and 10% at plasma concentrations of 4.6 and 12.6 nmol/L, respectively. The accuracy of measurements for samples above the calibration range was assessed with a 10-fold dilution of plasma to which androstenedione had been added (calculated amount, 258 nmol/L; recovery, 111%).

Data were analyzed by repeated-measures ANOVA for time-administration interactions (analyses of the difference in testosterone or androstenedione values between androstenedione and placebo treatment at each time point), which were found to be significant. Subsidiary tests were then applied to locate the differences.

The plasma concentration profiles of testosterone and androstenedione are shown in Fig. 1A and Fig. 1B, respectively; the differences between treatment and control values were highly significant for both hormones (P ≤0.0005). Subsidiary tests showed that androstenedione concentrations remained significantly different from control values from 15 min to 24 h, inclusive, and that testosterone concentrations remained significantly different from 30 min to 8 h, inclusive (for P values, see legend for Fig. 1). After administration, the plasma androstenedione concentration (Fig. 1B) reached a mean maximum value (SE) of 164 (19.5) nmol/L at 75 min, ~30-fold greater than the mean of the control values observed at that time [5.4 (0.8) nmol/L]; individual values for the area under the plasma concentration-vs-time curve (AUC) were approximately sevenfold greater (range, 2.4–12.5) than the corresponding values obtained after placebo administration. The mean plasma total testosterone concentrations increased to a maximum of 25.1 (3.3) nmol/L at 75 min compared with a mean control value of 0.3 (0.1) nmol/L (for each individual the maximum value observed was ≤1.5 nmol/L throughout the control period) and remained at supraphysiologic concentrations for up to 6 h. The AUC values for plasma testosterone concentration-vs-time curve were ~16-fold greater (range, 3.3–27.4) than the corresponding values obtained after placebo administration; the testosterone AUCs after androstenedione intervention ranged from 48.6 to 194 nmol·h/L. The ratios of the testosterone AUC to the androstenedione AUC (corrected for the corresponding areas for the placebo values), expressed as a percentage, yielded a mean value of 12.5% (range, 7.8–21.6%).

The mean exposure (as measured by AUC) to testosterone after androstenedione administration was more than one order of magnitude greater than the exposure during the control period. Furthermore, the mean ratio of the AUC of testosterone to androstenedione was very similar to the conversion ratio of 14% for blood-borne androstenedione to testosterone, as reported by Horton and Tait (7). The plasma testosterone concentrations observed were similar to those encountered in abuse of testosterone for anabolic purposes, the peak of the mean concentrations being at the upper end of the reference interval for men (10–35 nmol/L) (14). It is possible that a very small proportion of the measured testosterone could be attributable to cross-reactivity with 5α-dihydrotestosterone (DHT), particularly because plasma DHT in women is also likely to be greatly augmented with androstenedione administration. DHT binds with greater affinity to the androgen receptor (15) and hence is considered more potent than testosterone in androgenic target tissues. It would be of interest to measure the extent of changes in plasma DHT, in addition to other plasma hormones, as part of this ongoing study.

The acute hormonal response to ingestion of 100 mg of androstenedione in men produces a three- to fourfold increase in plasma androstenedione, as shown by King et al. (1), whereas our investigation in women showed a much larger increase. In that study, the plasma androstenedione appeared to plateau between ~2 to 4 h, whereas we observed a large decrease by 4 h after administration. Hence, although the time–concentration

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**Fig. 1.** Effect of oral administration of androstenedione (■) or placebo (○) at 0 h on mean plasma concentrations of testosterone (A) and androstenedione (B) in 10 healthy young women.

After androstenedione intervention, plasma testosterone concentrations were significantly different from control values at 30 min (P = 0.014) and at 45 min to 8 h, inclusive (P ≤0.002); plasma androstenedione concentrations were significantly different from control values at 15 min (P ≤0.05), 30 min, and 45 min (P ≤0.01) and at 1–24 h, inclusive (P ≤0.001). Error bars, SE. Note: SE values were too small to be displayed for the placebo intervention.
profiles may be different, the exposure to this dose of androstenedione may be similar. Unfortunately, the majority of publications to date that include data on the disposition of androstenedione do not detail changes in the AUCs of either androstenedione or testosterone as a measure of exposure. Assuming there is no large difference in steroid metabolic pathways between genders, then the difference in plasma androstenedione profiles observed may possibly be rationalized by differences in formulated products, but further investigations are required to support this supposition.

In conclusion, the marketing of androstenedione as a dietary supplement should be a cause for concern because even a single administration of 100 mg produced supraphysiologic concentrations of plasma testosterone in healthy women. The exposure to testosterone would be expected to be repeated with daily administration of 100 mg of androstenedione over several weeks, but in our opinion, the risk of virilization precludes testing this hypothesis. Given the concern raised by healthcare professionals regarding the medical consequences of misuse of anabolic steroids, not least that of virilization (16), we recommend that drug regulatory authorities consider classifying androstenedione with the same status as testosterone to control its sale.

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References


Comparison of Five Thrombin Time Reagents, Michele M. Flanders,1 Ronda Crist,1 and George M. Rodgers2,2

(1 ARUP Laboratories, Salt Lake City, UT 84108; 2 Departments of Medicine and Pathology, University of Utah Health Sciences Center, Salt Lake City, UT 84132; * address correspondence to this author at: Division of Hematology, University of Utah Health Science Center, 50 North Medical Dr., Salt Lake City, UT 84132; fax 801-585-5469, e-mail george.rogers@hsc.utah.edu)

The thrombin time (TT) assay is a core test in clinical laboratories that perform coagulation testing. This assay screens for abnormalities in the conversion of fibrinogen to fibrin. The time necessary for fibrinogen to clot is affected by hypofibrinogenemia, dysfibrinogenemia, and the presence of inhibitors of the fibrinogen-to-fibrin reaction (heparin, hirudin, fibrin degradation products, and paraproteins) (1). The TT is used primarily to evaluate plasma specimens with prolonged activated partial thromboplastin time (APTT) values and, to a lesser extent, prolonged prothrombin time (PT) values for hepatic or other thrombin inhibitors. The test is also useful to detect quantitative and qualitative fibrinogen abnormalities. Important criteria for selecting a TT reagent are high sensitivity to heparin, sensitivity to hypofibrinogenemia, and acceptable precision. We compared five commercial TT reagents on our automated coagulation analyzer and evaluated their sensitivity to fibrinogen and heparin concentration, their precision, and their accuracy.

Information on each of the five TT reagents evaluated is provided in Table 1 of the data supplement (available with the online version of this Technical Brief at http://www.clinchem.org/content/vol49/issue1/). Each commercial reagent was reconstituted according to the manufacturer’s instructions and used to measure TT in singleton measurements on the STA-R coagulation analyzer (Diagnostica Stago) according to the manufacturer’s specifications and laboratory standards. Clotting times for...