Acute hormonal response to sublingual androstenediol intake in young men

GREGORY A. BROWN, EMILY R. MARTINI, B. SCOTT ROBERTS, MATTHEW D. VUKOVICH, AND DOUGLAS S. KING

1Exercise Biochemistry Laboratory, Department of Health and Human Performance, Iowa State University, Ames, Iowa 50011; and 2Human Performance Laboratory, Department of Health, Physical Education, and Recreation, South Dakota State University, Brookings, South Dakota 57007

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Brown, Gregory A., Emily R. Martini, B. Scott Roberts, Matthew D. Vukovich, and Douglas S. King. Acute hormonal response to sublingual androstenediol intake in young men. J Appl Physiol 92: 142–146, 2002.—The effectiveness of orally ingested androstenediol in raising serum testosterone concentrations may be limited because of hepatic breakdown of the ingested androgens. Because androstenediol administered sublingually with cyclodextrin bypasses first-pass hepatic catabolism, we evaluated the acute hormonal response to sublingual cyclodextrin androstenediol supplement in young men. Eight men (22.9 ± 1.2 yr) experienced in strength training consumed either 20 mg androstenediol in a sublingual cyclodextrin tablet (Sl Diol) or placebo (Pl) separated by at least 1 wk in a randomized, double-blind, crossover manner. Blood samples were collected before supplementation and at 30-min intervals for 3 h after supplementation. Serum hormone concentrations did not change with Pl. Serum androstenedione concentrations were increased (P < 0.05) above baseline (11.2 ± 1.1 nmol/l) with Sl Diol from 60 to 180 min after intake and reached a peak concentration of 25.2 ± 2.9 nmol/l at 120 min. Serum free testosterone concentrations were increased from 86.2 ± 9.1 pmol/l with Sl Diol from 30 to 180 min and reached a peak concentration of 175.4 ± 12.2 pmol/l at 60 min. Serum total testosterone concentrations increased above baseline (25.6 ± 2.3 nmol/l) from 30 to 180 min with Sl Diol and reached a peak concentration of 47.9 ± 2.9 nmol/l at 60 min. Serum estradiol concentrations were elevated (P < 0.05) above baseline (0.08 ± 0.01 nmol/l) from 30 to 180 min with Sl Diol and reached 0.14 ± 0.02 nmol/l at 180 min. These data indicate that sublingual cyclodextrin androstenediol intake increases serum androstenedione, free testosterone, total testosterone, and estradiol concentrations.

METHODS

Approach to the problem. Although changes in serum hormones in men are observed within 2 h after ingestion of a weak androgen, the lack of meaningful increases in serum testosterone concentrations suggests a large hepatic catabolism of the weak androgens. Because sublingual delivery avoids first-pass clearance and cyclodextrins facilitate diffusion of an androgen across the oral mucosa, the presence of weak androgens is advertised to increase serum testosterone concentrations. However, increasing serum testosterone concentrations through the ingestion of weak androgens appears to be limited by hepatic catabolism of the ingested androgen. Although ~3% of serum androstenedione and ~15% of androstenediol can be converted to testosterone (5), as much as ~89% of orally administered androstenedione may be catabolized into glucuronides before it can enter the extraplanchnic circulation, and ~98% of testosterone formed from orally infused androstenedione undergoes hepatic extraction (15). Consistent with these findings, oral intake of 100–200 mg androstenedione (6, 8, 11, 17, 19, 25) or androstenediol (6, 11) does not change serum testosterone concentrations in men. Therefore, a mode of androgen delivery that bypasses first-pass hepatic catabolism may enhance the conversion of a precursor steroid into testosterone and increase serum testosterone concentrations in men.

Androstenediol can be reversibly converted to testosterone, androstenedione, and dehydroepiandrosterone (5, 16). In addition to androgenic interconversion, the aromatization of these androgens produces the majority of serum estrogens in men (4, 21). The effect of androstenediol supplementation on serum estrogens in young men has not been previously explored.

A cyclodextrin compound is a cyclic oligosaccharide formed from the enzymatic degradation of starch that forms an inclusion complex with steroid hormones, facilitating the passage of the steroid across the oral mucosa while the ingested cyclodextrin undergoes digestion (10, 28). The sublingual delivery of a hydroxypropyl-β-cyclodextrin compound containing small doses of testosterone produces an approximately fivefold increase in serum testosterone concentrations in men (24, 26, 28). Because the hormonal responses to sublingual cyclodextrin androstenediol have not been previously examined, the purpose of this investigation was to evaluate the acute serum hormonal response to sublingual cyclodextrin androstenediol in young men.

Address for reprint requests and other correspondence: D. S. King, Dept. of Health and Human Performance, 248 Forker Bldg., Iowa State Univ., Ames, IA 50011 (E-mail: dsking@iastate.edu).

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or lack of effectiveness of sublingual cyclodextrin androstenediol in increasing serum testosterone as well as other hormones should be evident within 3 h of intake.

Subjects. Eight men experienced in strength training (age 22.9 ± 1.2 yr; height 180.6 ± 2.0 cm; weight 83.0 ± 2.0 kg; body fat 12.7 ± 1.7%) volunteered to participate in this study. Subjects completed a written medical history to eliminate persons with a known chronic disease, abuse of alcohol, or use of illicit drugs. Subjects also completed a questionnaire regarding exercise history and use of nutritional supplements. Before participation in this project, approved by the Iowa State University Human Subjects Review Board, all subjects signed an informed consent.

Blood sample collections. After an overnight fast, subjects reported to the laboratory between 5:30 and 7:00 AM. After insertion of a flexible catheter into an antecubital vein, blood samples were collected without stasis from sitting subjects 20 min before supplementation, immediately before supplementation, and at 30-min intervals for 3 h after supplement administration. Blood samples were allowed to clot in an ice bath until centrifugation and serum separation.

Dietary control. Subjects were instructed to eat their normal diet and record dietary intake and exercise for 3 days before the initial trial and repeat the same diet and exercise protocol for the subsequent trial. Dietary records were analyzed by using commercial software (Nutritionist 4, N-Squared computing, San Bruno, CA).

Supplementation. Subjects were administered either placebo (rice flour; Pl) or sublingual hydroxypropyl-β-cyclodextrin androstenediol (Sl Diol) in a randomly assigned, double-blind, crossover manner separated by a period of at least 7 days. Supplements were administered as an unmarked white capsule that was swallowed (Pl) or an unmarked white tablet that was placed under the tongue and allowed to completely dissolve (Sl Diol). Subjects and research assistants were informed that the purpose of the study was to evaluate the effectiveness of either an ingested or sublingual supplement. The Sl Diol (Cyclo-Diol, Kaizen, Los Angeles, CA) was analyzed for hormone content using HPLC (Integrated Biomolecule, Tuscon, AZ), and each tablet contained 21.4 mg androstenediol, 3.7 mg androstenedione, and no other steroid hormones.

Body composition. Hydrostatic weight was determined by using a computer-interfaced load cell. After estimation of residual volume from the measurement of maximal voluntary lung capacity (13) by using a computerized spirometer (PC Flow+ for Windows, Spirometrics Medical Equipment, Grey, ME), percent body fat was calculated by using the Siri equation (27).

Hormonal analysis. Serum concentrations of total and free testosterone and androstenedione, and estradiol were measured in duplicate via commercial tracer-analog RIA (Coat-A-Count, Diagnostic Products, Los Angeles, CA). When the serum hormone concentrations exceeded the highest calibrator value provided with the RIA kit, the sample was reanalyzed according to the manufacturer’s instructions by diluting the sample with reagent containing no hormone. The accuracy of the RIA kits was verified by analyzing samples of known hormone concentrations. The intra-assay coefficients of variation for total testosterone, free testosterone, androstenedione, and estradiol were 5.9, 5.8, 5.2, and 6.1%, respectively. No data on the cross-reactivity of androstenediol with the other steroid hormone assays are available. According to the manufacturer of the RIA kits, cross-reactivity is <0.5%, except for a 1.49% cross-reactivity of the androstenedione assay for testosterone.

Calculations and statistics. Data were analyzed by using commercial software (SPSS, Chicago, IL). Statistical analyses were performed using a two-factor (supplement by time) repeated-measures ANOVA. Specific mean differences were analyzed with a Student-Newman-Keuls post hoc test. Data are presented throughout the text as means ± SE. Percent changes in hormones were calculated as the mean increase above baseline during the 3 h after supplement intake. Area under the curve (AUC) was calculated by using the trapezoidal model. Peak hormone concentrations were defined as the numerically highest concentrations reached for each subject during the time course of the study.

RESULTS

Subjects. All subjects were students at Iowa State University and reported participation in a full-body resistance-training program for 6.0 ± 1.1 yr. Subjects participated in resistance training 3.6 ± 0.5 days/wk for 1.6 ± 0.2 h/exercise session. Five subjects reported regular participation in aerobic exercise 3.0 ± 0.5 days/wk for 20 ± 3.2 min/session. Although no subjects reported participation in competitive weight lifting or bodybuilding, one subject was a collegiate track athlete. None of the subjects reported any current or previous use of anabolic steroids, only two subjects reported previous use of nutritional supplements (creatinine n = 2, “weight gain powder” n = 1), and no subject consumed any supplements during the 9 mo before study.

Supplementation. Dissolution of Sl Diol required 12.4 ± 1.4 min after the tablet was placed sublingually.

Dietary intake. Subjects reported a mean daily energy intake of 8,770 ± 904 kJ/day for 3 days before the initial trial, and they repeated the same diet for the subsequent trial. Daily dietary intake was 161 ± 60 g/day (26 ± 6%) protein, 268 ± 49 g/day (46 ± 6%) carbohydrate, and 68 ± 9 g/day (37 ± 3%) fat.

Hormonal response. Serum hormone concentrations did not change in Pl. Serum androstenedione concentrations were increased from 60 to 180 min in Sl Diol (Fig. 1; P < 0.05), whereas peak concentrations of

Fig. 1. Serum androstenedione concentrations after administration of placebo (Pl) or sublingual cyclodextrin androstenediol (Sl Diol).

SL-DIOL

PL

*Different from 0 and −20 min, P < 0.05.

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24.3 ± 1.5 nmol/l were observed 150 min after administration. The AUC for serum androstenedione concentrations was higher \((P < 0.05)\) in Sl Diol (1,604.9 ± 136.3 nmol·l\(^{-1}·\text{min}^{-1}\)) compared with Pl (96.0 ± 50.0 nmol·l\(^{-1}·\text{min}^{-1}\)).

Sl Diol increased serum free testosterone concentrations (Fig. 2; \(P < 0.05\)) from 30 to 180 min after administration, whereas peak concentrations (175.4 ± 2.2 pmol/l) were observed 60 min after supplementation. The AUC for serum free testosterone concentrations was higher \((P < 0.05)\) in Sl Diol (11,137 ± 1,573 pmol·l\(^{-1}·\text{min}^{-1}\)) compared with Pl (750 ± 218 pmol·l\(^{-1}·\text{min}^{-1}\)). Serum free testosterone concentrations increased in all subjects in response to Sl Diol.

Sl Diol intake increased serum total testosterone concentrations \((P < 0.05; \text{Fig. 3})\) above basal (25.6 ± 2.3 nmol/l) from 30 to 180 min and peak serum total testosterone concentrations were observed at 60 min (47.9 ± 2.9 nmol/l). The AUC for total testosterone was higher \((P < 0.05)\) in Sl Diol (2,673 ± 577 nmol·l\(^{-1}·\text{min}^{-1}\)) compared with Pl (204 ± 108 nmol·l\(^{-1}·\text{min}^{-1}\)). Serum total testosterone concentrations were elevated in all subjects after Sl Diol intake. The calculated effect size for the comparison of basal total testosterone concentrations to those observed at 60 min was large (0.89), emphasizing the effect of Sl Diol intake on serum total testosterone concentrations.

Serum estradiol concentrations were increased above basal (0.08 ± 0.01 nmol/l) 30–180 min after administration in Sl Diol \((P < 0.05; \text{Fig. 4})\) and reached 0.14 ± 0.02 nmol/l 180 min after Sl Diol administration. The AUC for serum estradiol concentrations was greater \((P < 0.05)\) for Sl Diol (5.26 ± 0.86 nmol·l\(^{-1}·\text{min}^{-1}\)) compared with Pl (1.12 ± 0.44 nmol·l\(^{-1}·\text{min}^{-1}\)).

**DISCUSSION**

The main finding of this study is that ~20 mg cyclodextrin androstenediol taken sublingually increases serum testosterone and estradiol concentrations in healthy young men. Because a single 200-mg dose of androstenediol taken orally does not change serum free or total testosterone concentrations in young men (11), the present findings suggest that sublingual cyclodextrin is superior to oral ingestion for the delivery of androstenediol to the peripheral tissues and subsequent conversion to testosterone.

Sl Diol contained both androstenediol (21.4 mg/tablet) and androstenedione (3.7 mg/tablet). Because both androstenedione and androstenediol can be converted to testosterone (5, 14, 16), it is possible that the increases in serum testosterone were at least partially due to the conversion of androstenedione, and not androstenediol, to testosterone. However, serum testosterone concentrations were elevated at 30 min after intake, whereas serum androstenedione concentrations were not elevated until 60 min after intake. Furthermore, we have observed that increases in the serum androstenedione concentration of a magnitude greater than that observed in the present study do not increase serum testosterone concentrations (8, 17). Therefore, the increased testosterone concentrations are likely due solely to the androstenediol contained in Sl Diol.
Sublingual cyclodextrin testosterone intake results in peak serum testosterone concentrations 20–30 min after intake (26, 28), whereas SI Diol results in peak serum testosterone concentrations ~60 min after intake. In hypogonadal men, a 25- to 10-mg dose of cyclodextrin testosterone administered sublingually causes serum testosterone concentrations to reach supranormal levels (~35–85 nmol/l) within 60–90 min. Serum testosterone concentrations in these men return rapidly to values below the normal range within 3 h and return to baseline concentrations by 6 h with a half time of ≤2 h (24, 26, 28), suggesting that effective therapy with sublingual cyclodextrin testosterone would require administration every 2 h (28). Because serum testosterone concentrations after SI Diol intake remained ~40% above baseline at 180 min, it is unknown how long serum testosterone concentrations remain elevated after cyclodextrin androstenediol intake in eugonadal men. However, with allowance made for the 30- to 40-min delay in the testosterone response after SI Diol, the change in serum testosterone concentrations is similar to the pattern exhibited after sublingual cyclodextrin testosterone intake (24, 26, 28). Therefore, it is likely that sustained increases in serum testosterone with SI Diol would also require frequent dosing.

Androstenediol intake is advertised to enhance muscle mass and strength gains during resistance training. However, chronic ingestion of androstenediol does not enhance the adaptations to resistance training in men (6). In contrast to oral androstenediol ingestion (6, 11), SI Diol increased serum testosterone concentrations. It is currently unknown whether a transient increase in serum testosterone of the magnitude elicited by SI Diol intake alters muscle mass or strength.

The tracer-analog method for measuring free testosterone has been criticized recently on the basis of findings of a positive relationship between sex hormone-binding globulin and measured free testosterone concentrations (30). In addition, it has been stated that this method detects a constant fraction of the total testosterone concentrations, rather than the testosterone that is free from binding proteins (30). However, others have concluded that the tracer-analog method accurately measures free testosterone concentrations (18, 23). The finding that both free and total testosterone concentrations are significantly increased after SI Diol intake suggests that androstenediol taken sublingually is converted to testosterone.

As previously observed with sublingual cyclodextrin testosterone intake (26, 28), SI Diol increases serum estradiol concentrations, suggesting that a significant portion of the increased serum testosterone and/or exogenous androgen underwent aromatization (20). Oral ingestion of androstenedione or androstenediol increases serum estrogens but not serum testosterone concentrations (6–8, 17, 19, 25), indicating aromatization of the weak androgen. In the present study, it was not possible to determine whether the elevated serum estradiol concentrations result from the exogenous androstenediol, or from the serum testosterone formed from the androstenediol, or both. On the basis of the findings with ingested androstenedione (6–8, 17, 19, 25) and with sublingual cyclodextrin testosterone (26, 28), the increased serum estradiol concentrations associated with SI Diol are likely to remain elevated for a longer period of time than will testosterone. Furthermore, we have recently observed that the increases in serum estrogens with chronic androstenediol intake are larger than the increases in serum testosterone (7a). These findings may be clinically significant, because elevated estradiol concentrations are associated with benign prostate hypertrophy (15) and gynecomastia (4).

In contrast to the high individual variability in the hormonal response to androstenedione ingestion (19), all of the subjects exhibited similar increases in serum testosterone, androstenedione, and estradiol concentrations in response to SI Diol intake. Salehian et al. (26) observed that the hormonal response to sublingual cyclodextrin testosterone was not different between the first dose and subsequent doses during a 60-day treatment regimen, indicating that there is no change in the absorption of a sublingually administered androgen with prolonged use. In contrast, there is a reduction in the hormonal response to prolonged ingestion of androstenedione (6, 8, 17) and dehydroepiandrosterone (9, 22), suggesting either reduced entry into or enhanced clearance of the androgen from the circulation, or an alteration in the enzymatic transformation of weak androgens into other hormones in response to prolonged intake. Thus, although it appears that a single dose of SI Diol produces a uniform hormonal response in young strength-trained men, the hormonal response to prolonged use of SI Diol is unknown.

Chronic oral intake of androstenediol lowers serum high-density-lipoprotein cholesterol concentrations (6, 7a). Chronic androstenediol intake also elevates serum dihydrotestosterone concentrations (7a), which, combined with elevated estradiol concentrations, may also contribute to benign prostate hypertrophy (29). Increased serum concentrations of androstenedione have been associated with pancreatic cancer in men (12). The effects of a transient increase in serum testosterone concentrations have not been evaluated. Whereas low endogenous testosterone concentrations may be related to diabetes and hypertension (2, 3), increased serum testosterone concentrations may cause a deterioration of the blood lipid profile (1). These findings raise the possibility that more long-term use of sublingual androstenediol may pose significant health risks.

In conclusion, the use of a sublingual cyclodextrin androstenediol nutritional supplement causes rapid and transient increases in serum testosterone concentrations. SI Diol also causes increases in serum estradiol and androstenedione concentrations. The effects of the altered hormonal milieu observed after SI Diol intake on health or the adaptations to resistance training are not known.
REFERENCES


