Four weeks of androstenedione supplementation diminishes the treatment response in middle aged men

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METHODS

Subjects

Eight healthy men volunteered to participate in this study which was approved by the Institution’s human research review committee. Before the study, each subject completed and signed a medical history and consent form that described the study protocol and potential risks and discomforts. Baseline characteristics were mean (SD) age 44.1 (3.0) years (range 40–48), body mass 76.3 (9.4) kg, and height 175 (5.3) cm. Only two subjects participated in a resistance training programme; both had participated for five years or more. For the duration of the trial, all subjects agreed not to change the frequency, intensity, or volume of their exercise programme, or their dietary habits.

Study design

A randomised, double blind, cross over, placebo controlled design was used to assess subject responses to supplemented and placebo treatment conditions. A cross over design was chosen to control for initial differences in hormonal concentrations and the effects this may have on individual responses to supplementation. Each subject was tested at the beginning (day 0) and end of a 28 day supplementation period with 200 mg/day androstenedione increases AUC for serum androstenedione in the day 0 condition, continued supplementation is characterised by a diminished treatment response, coupled with time 0 increases in testosterone precursors but not testosterone.

Anstofisterone is marketed to middle aged (> 40 years) men to ameliorate the effect of aging on the hypothalamic pituitary gonadal axis. Some studies of male androgenic function report no decline in serum total testosterone concentration with age, whereas others report decreasing concentration at age 45–50 years that may be within the normal range for young men. As androstenedione is a testosterone precursor, and supplementation has been shown to raise serum androstenedione concentration, it is plausible that elevated androstenedione concentrations could result in increased conversion of androstenedione into testosterone, especially in an aging population. Several studies have reported that supplementation with 200–300 mg/day androstenedione can produce acute and chronic increases in total and free testosterone concentrations, whereas others have reported no changes in younger (<40 years) and middle aged (>40 years) men. These studies used different dosages, dosing patterns, lengths of supplementation, as well as, a variety of age groups, which may contribute to these inconsistent findings.

Of perhaps greater concern is the potential for alterations in hormonal balance with prolonged supplementation. Strong regulatory mechanisms exist that alter the production and conversion of hormones after prolonged administration of androgens. The purpose of this study was to determine if baseline (time 0) and pharmacokinetic hormonal responses to androstenedione supplementation are altered after four weeks of supplementation with 200 mg/day androstenedione in middle aged men.

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ORIGINAL ARTICLE

See end of article for authors’ affiliations

Correspondence to:
Assistant Professor
Beckham, Department of Kinesiology, University of Texas at Arlington, Box 19259, Arlington, TX 76019, USA;
sbeckham@worldnet.att.net

Abbreviations: DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone binding globulin; AUC, area under the curve; LDL, low density lipoprotein; HDL, high density lipoprotein; VLDL, very low density lipoprotein; POMS, profile of mood state

mg/day androstenedione and a placebo. Figure 1 shows a schematic of the testing protocol. A 28 day washout period was used between the two supplementation periods. During both treatments, subjects were examined to determine hormonal concentrations at time 0, as well as a 180 minute pharmacokinetic response to each treatment.

**Treatment**

The treatments consisted of 200 mg/day androstenedione (two 100 mg tablets) or two tablets of a rice powder placebo, administered each morning. Each treatment was distributed in bottles that were individually coded with a random number sequence so that, in the case of an adverse event, the code could be broken without sacrificing the integrity of the remaining cohort. All supplements were of the same lot and provided by Metabolic Response Modifiers (Newport Beach, California, USA). A certificate of analysis from the company reported the product to be 95% pure.

**Cardiovascular and anthropometric testing**

Subjects reported to the laboratory after a 12 hour fast and abstinence from exercise for 24 hours. Data were collected at about the same time each visit to control for diurnal variations in certain hormones. Resting heart rate and blood pressure measurements were taken at the beginning of each testing session. Subjects were then weighed for total body mass before determination of body density by hydrostatic weighing. The three highest underwater weights at each visit were averaged to obtain underwater weight. Underwater weights were corrected for residual volume using an automated multiple breath oxygen dilution procedure with a Vmax series 229 metabolic analyser (SensorMedics, Yorba Linda, California, USA). The Siri equation was used to convert body density into percentage body fat. Next, waist and hip circumferences were measured using a Gulick tape. Lastly, subjects completed a questionnaire designed by the researchers at each visit that enquired about androgenic effects typically associated with steroid use.

**Blood lipid assessment**

Blood lipid indices were measured before the pharmacokinetic assessment from a finger stick (Cholestech LDX System; Cholestech LDX, Hayward, California, USA) to determine total, low density lipoprotein (LDL), high density lipoprotein (HDL), and very low density lipoprotein (VLDL) cholesterol, total cholesterol/HDL cholesterol ratio, and triglycerides. The intra-assay and interassay coefficients of variation for the analysis were 2.5% and 3.1% for total cholesterol, 4.1% and 5.4% for HDL cholesterol, 2.6% and 2.8% for VLDL cholesterol, and 2.6% and 2.8% for triglycerides.

**Pharmacokinetic and baseline hormone assessments**

After a fasting assessment for lipid indices, the time 0 and pharmacokinetic response for the 180 minute period after ingestion of a placebo or androstenedione were assessed. As
the pharmacokinetic assessment required an additional four hours to complete the data collection process, subjects were permitted to eat a snack. Meikle et al. report that ingestion of a high fat meal (57% fat) produced acute reductions in total and free testosterone, but ingestion of a low fat/mixed carbohydrate/protein meal did not; therefore subjects were permitted to ingest a low fat (<20% fat)/mixed carbohydrate/protein energy bar and consume a carbohydrate sports drink (Gatorade) after the cholesterol testing and before the pharmacokinetic assessment. Analysis of testosterone concentrations for the placebo trials by repeated measures analysis of variance (p<0.05) failed to show any significant differences across the 180 minute sampling period. This finding supports the assumption that the low fat/mixed carbohydrate/protein meal had no effect on testosterone levels.

Before the pharmacokinetic trial, subjects were fitted with a 21 gauge catheter that was inserted into an antecubital vein. After a 30 minute habituation period, the first blood sample was collected at time 0. Supplements were then given orally, and blood samples were collected every 30 minutes for 180 minutes. Blood samples were collected in a plain tube, allowed to clot for 20 minutes, and spun in a centrifuge at 4°C. Serum was then pipetted into a storage tube and stored at −70°C. At a later date, serum androstenedione was analysed in duplicate in antibody coated tubes with radioimmunoassay kits (Diagnostic Products Corporation, Los Angeles, California, USA). Total testosterone, dehydroepiandrosterone sulfate (DHEAS), oestradiol 17 (oestradiol), and sex hormone binding globulin (SHBG) concentrations were assessed using a solid phase, chemiluminescent immunoassay (Immulite; Diagnostic Products Corporation). With this system, the lowest detectable oestradiol concentration is 73.4 pmol/l. Therefore oestradiol values <73.4 pmol/l were recorded as 73.4 pmol/l for the purpose of this study. The intra-assay and inter-assay coefficients of variation for the analysis were 5.7% and 9.8% for total testosterone, 9.3% and 10.6% for oestradiol, and 6.5 and 8.7% for SHBG.

**Psychometric assessment**

All subjects completed a profile of mood state (POMS) questionnaire at each visit. The POMS measures dimensions of affect or mood in six categories. A Wilcoxon signed rank test (p<0.05) was used to determine if total scores were significantly different between trials. Experiment wise error rate for the POMS was calculated for the six categories as 1−(1−0.05)° = 0.264, where the iteration “6” is the number of categories in the POMS assessment. Subsequently, 0.264 was divided by six so that the α level for determining significance was p = 0.044 of self reported behaviours.

**Statistical analysis**

Integrated area under the curve (AUC) above time 0 concentrations and the associated metabolic clearance rate (litres/h) for androstenedione were determined by trapezoid calculation for the 180 minutes after supplementation for each analyte at each of the four testing conditions (PK Solutions Software 2.0, Montrose, Colorado, USA). The following equation was used to calculate systemic clearance rate (CL) for the observed AUC (0-1):

\[ CL = \frac{F \times D}{AUC_{0-1}} \]

where F = fraction of dose absorbed and D = dose. A repeated measures multivariate analysis of variance (p<0.05) with a Fisher’s least squares difference post hoc analysis was used to compare AUC for the testing conditions (Statview, Cary, North Carolina, USA). As no statistical difference was observed between the two placebo conditions, results from the first placebo visit (day 0) were used in the analysis of all analytes. A repeated measures multivariate analysis of variance (p<0.05) with a Fisher’s least squares difference post hoc analysis was also used to compare time 0 hormonal concentrations measured at the beginning of each test session. A repeated measures analysis of variance (p<0.05) was used to assess concentration differences across time for analytes that showed significant changes for either time 0 or AUC values compared with placebo. In addition, a Pearson product-moment correlation was computed to assess the relations between AUC for various hormonal responses. A repeated measures multivariate analysis of variance (p<0.05) was also used to examine changes related to body composition (body fat percentage) and other body measures multivariate analysis of variance (p<0.05) was also used to examine changes related to body composition (body fat percentage) and other body measures.
mass, body fat, and waist/hip ratio), resting cardiovascular data (resting heart rate, systolic and diastolic blood pressure), and the lipid profile (total, LDL, HDL, and VLDL cholesterol, total cholesterol/HDL cholesterol ratio, and triglycerides).

RESULTS

Pharmacokinetics (AUC)

Figures 2 and 3 depict integrated AUC concentrations for each analyte during the 180 minute period after administration of androstenedione or a placebo. The serum androstenedione concentration (AUC) was significantly higher after the first administration of 200 mg androstenedione (day 0) compared with both placebo and day 28 of supplementation. However, AUC concentrations were not significantly different between any of the treatments for other hormones. Furthermore, no differences were noted between androstenedione clearance rates for AUC at day 0 compared with day 28 of supplementation.

Correlations of AUC examining the relation between androstenedione and total testosterone were $r = 0.66$ (p = 0.08) and $r = 0.79$ (p = 0.01) for day 0 and day 28 responses respectively. AUC for androstenedione correlated strongly with DHEAS ($r = 0.871$, p<0.005) on day 0 of androstenedione supplementation but not on day 28 ($r = 0.59$, p = 0.1). In addition, AUC for total testosterone and DHEAS correlated significantly for both the day 0 ($r = 0.89$, p = 0.001) and day 28 ($r = 0.87$, p<0.005) supplemented conditions.

Time 0 hormonal concentrations

The repeated measures multivariate analysis of variance for time 0 hormonal concentrations showed significant differences (p = 0.01) between treatments (table 1). Time 0 androstenedione concentrations increased by 215% after 28 days of supplementation compared with placebo (p = 0.08). DHEAS (time 0) concentrations were significantly elevated after 28 days of supplementation (p = 0.0002). No significant differences in concentrations for total testosterone, oestradiol, or SHBG were found between treatments.

Time series

Statistical analysis of androstenedione concentrations across time showed significant treatment (p<0.0001), time (p<0.0001), and time × treatment interaction (p<0.0001) effects (fig 4). Beginning at 90 minutes after administration, serum androstenedione concentrations were significantly higher for both the day 0 (p<0.05) and day 28 (p<0.05) supplemented trials compared with placebo. The only exception was minute 120 when only day 0 (p<0.005) but not day 28 (p = 0.07) concentrations were higher than placebo. For DHEAS, the repeated measures analysis of variance showed a significant treatment (p<0.005), time (p = 0.01), and treatment × time interaction (p = 0.001) effect. Figure 4 also depicts the DHEAS concentrations across time for all three conditions. As noted above, DHEAS concentrations were significantly higher at time 0 after 28 days of androstenedione supplementation compared with day 0 and placebo (p<0.001). This trend continued throughout the 180 minute sampling phase. DHEAS concentrations on day 0 were significantly higher than placebo (p = 0.009) only at 180 minutes after administration.

Resting cardiovascular and anthropometric data, lipids, and POMS

Body composition, body mass, and waist/hip ratio did not change significantly (p = 0.95) after four weeks of supplementation with androstenedione. Furthermore, no significant

<table>
<thead>
<tr>
<th>Condition</th>
<th>Androstenedione, day 0</th>
<th>Androstenedione, day 28</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androstenedione (nmol/l)</td>
<td>5.2 (0.3)</td>
<td>16.4 (7.0)†</td>
<td>5.6 (0.4)</td>
</tr>
<tr>
<td>DHEAS (µmol/l)</td>
<td>7.0 (0.4)</td>
<td>12.9 (1.3)‡</td>
<td>7.0 (0.8)</td>
</tr>
<tr>
<td>Total testosterone (nmol/l)</td>
<td>21.0 (1.9)</td>
<td>24.0 (3.3)</td>
<td>21.0 (3.2)</td>
</tr>
<tr>
<td>Oestradiol-17β (pmol/l)</td>
<td>138.4 (6.2)</td>
<td>130.7 (11.3)</td>
<td>121.1 (10.2)</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>29.9 (4.7)</td>
<td>24.4 (4.0)</td>
<td>28.3 (4.7)</td>
</tr>
</tbody>
</table>

Values are mean (SE).

*Significantly different from placebo (p = 0.08).
†Significantly different from day 0 (p = 0.07).
‡Significantly different from day 0 and placebo (p = 0.0002).

DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone binding globulin.
It is difficult to interpret these findings because of differences in the age of subjects, length of supplementation, dose, dosing pattern, type of response measured (acute compared with chronic changes), and the specific method used to assess acute responses (AUC compared with change at different time points). These studies suggest that short term (0–7 days) supplementation with 200–300 mg/day androstenedione may produce acute increases in AUC for total and free testosterone in younger men,11 but not when analysed as changes across time points.6 Only one study reported a chronic increase in free testosterone concentration after four weeks of supplementation in younger men. With regard to middle aged men, 200–300 mg/day androstenedione administration for 4–8 weeks has been shown to temporarily elevate free and/or total testosterone levels.7,6 Broeder et al.,11 however, reported that total and free testosterone concentrations returned to baseline after 12 weeks of supplementation. No other studies have examined the acute response to supplementation in older men.

Our study used integrated AUC to assess the acute response to supplementation because it provides a better appreciation of the appearance, metabolism, and clearance of a hormone as contrasted with individual time points or total AUC. We found a slight (p = 0.23) increase in AUC for total testosterone in the day 0 condition, which was 143% and 148% greater than the placebo and day 28 trials respectively. In a similar pattern to androstenedione, the pharmacokinetic response for total testosterone in the day 28 condition decreased by 60% as compared with the day 0 condition. The strong correlations between androstenedione and total testosterone are consistent with this finding. Furthermore, time 0 values for total testosterone were not significantly elevated after four weeks of supplementation. Failure of androstenedione ingestion to produce a significant increase in total testosterone concentrations may be an integrated function of several metabolic regulatory areas. These areas include increased clearance rate for testosterone,2 10 11 declines in secretion of luteinising hormone,7 and conversion into other hormones such as oestradiol.6,8

It has been estimated that about 50% of oestradiol entering the blood de novo is produced by the aromatisation of testosterone.2 Therefore, even small increases in testosterone could conceivably increase oestradiol concentrations, particularly in men. Studies that used 100–300 mg/day supplementation of androstenedione reported significant increases in fasting and AUC concentrations for oestrone and oestradiol.7,6,10 12 13 The results of our study, however, were not consistent with these findings, as no significant differences were found for AUC or time 0 concentrations of oestradiol.
between any of the conditions. The fact that mean oestradiol concentration at time 0 for placebo was slightly lower than the other two conditions cures two to the greater integrated AUC values for the placebo condition. Examination of individual oestradiol responses showed varied response patterns for AUC. Another factor that may have limited the sensitivity of oestradiol measurements was the method of analysis, as the lowest detectable concentration of oestradiol is 73.4 pmol/l with the chemiluminescent immunnoassay. Moreover, out of the eight subjects in our study had oestradiol concentrations below 73.4 pmol/l (73.4–183.6 pmol/l is considered the normal range), and three showed values above 183.6 pmol/l at one or more times across the 180 minute sampling periods.

In addition to changes in hormonal concentrations, androgen administration may alter the concentration of SHBG, the primary transport protein for testosterone, dihydrotestosterone, and to a lesser extent oestradiol. Physiological changes in the concentration of SHBG can alter clearance rate, and hence, the amount of free (non-protein) versus protein bound steroid. Plasma SHBG concentrations are sensitive to changes in the circulating androgen/oestrogen ratio and have been shown to increase with oestrogen and decrease with androgen administration. In our study, we found that acute ingestion of androstenedione decreased SHBG AUC on day 0 and day 28 of androstenedione supplementation by 28% and 50% respectively compared with placebo; however, neither of these changes were significant. Consistent with our findings, Wallace et al. and Broder et al. did not report any significant changes in SHBG concentration. To the contrary, Leder et al. reported a significant decrease in mean daily baseline SHBG concentrations after seven days of supplementation with both 100 and 300 mg/day. The physiological significance of changes in SHBG concentrations with supplementation is unclear. Studies suggest that non-protein bound testosterone may underrepresent the biologically active portion in plasma. Furthermore, Brown et al. reported that supplementation can alter the concentration of dihydrotestosterone, which may have a higher binding affinity for SHBG than testosterone. In our study, plasma SHBG AUC did not correlate significantly with AUC for androgens such as androstenedione, total testosterone, and DHEAS, suggesting that changes in SHBG cannot be explained on the basis of the effect of these androgens.

An interesting finding in our study was a change in hormonal concentrations upstream to androstenedione and testosterone. DHEAS concentrations increased by 84% at time 0 after 28 days of supplementation with androstenedione (p = 0.09). This pattern is consistent with those observed by Broder et al. Also, as seen for androstenedione and total testosterone, the AUC response for DHEAS showed a tendency to decrease by the same proportion (58%) as observed for androstenedione and total testosterone after 28 days of supplementation. These changes in the relation between AUC for androstenedione and the DHEAS responses from day 0 to day 28 were significantly correlated (r = 0.86; p = 0.003). In addition, the strong correlations between AUC for total testosterone and DHEAS, as well as androstenedione and DHEAS, suggest that changes in these hormones are related.

Most (90%) of the circulating DHEAS is secreted by the adrenal gland and then converted into more potent androgens. As dehydroepiandrosterone (DHEA) and DHEAS are produced upstream of androstenedione, changes in DHEAS concentrations may be related to alterations in adrenal secretion, metabolism, or excretion and/or enzyme activity. The addition of androstenedione, as well as testosterone, to tissue preparations from human testis has been shown to inhibit the Δ,3β-hydroxysteroid dehydrogenase activity for dehydroepiandrosterone. Inhibition would tend to reduce the conversion of DHEA into androstenedione, thereby increasing serum concentrations of DHEA. As DHEA is readily and reversibly converted into its sulfate conjugate, DHEAS concentrations would also be expected to increase. Consistent with this finding, patients with Δ,3β-hydroxysteroid dehydrogenase deficiency exhibit elevated plasma concentrations of DHEAS.

With regard to the effect of androstenedione supplementation on the lipid profile, no changes were noted during our study. However, a 13% reduction in HDL cholesterol (p = 0.21) was noted in our trial. Despite the observation that this was not statistically significant, similar reductions in HDL cholesterol have been reported in other studies in which 200–300 mg/day was administered. The dosing pattern (one dose of 200 mg/day or two doses of 100 mg) used in this study may not be adequate to produce significant changes in HDL cholesterol with only 28 days of supplementation. In addition, the statistical power (0.23) for this analysis was low, which increases the probability of a type II error. The lipid profile was a secondary outcome measure, while the hormonal responses to supplementation were the primary outcome measure. Thus future investigations using similar research designs should take this into account.

Lastly, our study confirms those of others by showing that androstenedione does not affect anthropometric indices. These include percentage body fat, body mass, and waist/hip ratio. Neither does it appear to alter resting heart rate, blood pressure, or mood state. Two subjects did report an increase in mood swings and aggression/hostility and another reported an increase in hair growth after four weeks of androstenedione supplementation. Otherwise, there were no personal reports of other changes typically associated with steroid administration, such as acne or sex drive for either the androstenedione or placebo trials.

Androgen/pro hormone supplements have not been shown to enhance performance, favourably alter body composition, or positively affect various parameters associated with good health in younger or middle aged men. The potential for alterations in endogenous hormonal concentrations, including elevated concentrations of weaker androgens upstream of androstenedione is a concern as the long term effect in previously healthy men is unknown. Interestingly, the pharmacokinetic response to androstenedione supplementation is diminished after only 28 days of supplementation. Lastly, this study also provides no evidence that supplementation with 200 mg/day androstenedione will significantly elevate testosterone concentration. This study raises further concerns that androstenedione supplementation may lead to alterations in the hormonal profile that diminish the acute effects of supplementation and elevate baseline concentrations of hormones upstream of testosterone.

Take home message

Androstenedione supplementation in middle aged men did not produce significant increases in total testosterone but elevated the concentration of DHEAS, upstream of androstenedione. Furthermore, the acute pharmacokinetic response to supplementation was diminished after 28 days of supplementation. These findings raise health concerns associated with alteration of the endogenous hormonal profile.

Authors’ affiliations
S G Beckham, University of Texas at Arlington, USA
C P Ernest, Cooper Institute for Aerobics Research, Texas, USA

REFERENCES


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Group email for BASEM members

Following the last Executive meeting, it was decided that the Communications Officer, Dan Lane, should attempt to put all BASEM members with email on a group list in order that they can communicate more readily on both clinical and non-clinical matters. It will not mean that members will receive hundreds of irrelevant emails, but it will enable vastly improved communication throughout the Association.

If you would like to join the list, please forward your email address to Dan Lane (danclane@aol.com)