Acute hormonal responses to oral androstenedione in college-aged women

Joshua Carter Dewey
Iowa State University

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Acute hormonal responses to oral androstenedione in college-aged women

by

Joshua Carter Dewey

A thesis submitted to the graduate faculty in partial fulfillment of the requirements for the degree of

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Program of Study Committee:
Douglas King, Major Professor
Marian Kohut
Lee Alekel

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Graduate College
Iowa State University

This is to certify that the Master's thesis of

Joshua Carter Dewey

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy
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ABSTRACT

The following study examined the acute hormonal response to androstenedione ingestion in eight college-aged females. Between days three and five of the follicular phase and between 6am and 9am, subjects ingested placebo (100 mg rice pill), 100, or 300 mg of androstenedione in a random, double-blind, cross-over manner. Blood samples were collected before and every 30 min for 240 min post ingestion. Androstenedione ingestion increased serum androstenedione concentrations (basal 6.2 ± 0.8 nmol/L) at each time point from 60 to 240 min for both 100 mg (peak at 240 min = 22.6 ± 1.0 nmol/L) and 300 mg (peak at 210 min = 28.1 ± 1.3 nmol/L). Ingestion of 300 mg resulted in higher androstenedione concentrations at each time point from 120 to 240 min. Ingestion of androstenedione increased serum estradiol concentrations (basal 191 ± 24 pmol/L) only at 150 min with 100 mg (237 ± 35 pmol/L) and at each time point from 150 to 240 min with 300 mg (peak at 240 min = 260 ± 32 pmol/L). Androstenedione ingestion increased serum total testosterone concentrations (basal 1.2 ± 0.2 nmol/L) at each time point from 120 to 240 min (peak at 210 min = 5.5 ± 0.9 nmol/L) with 100 mg and from 60 to 240 min with 300 mg (peak at 210 min = 10.2 ± 1.6 nmol/L). Ingestion of 300 mg androstenedione caused a larger increase than the 100 mg at each time point from 120 to 240 min. These data suggest that androstenedione ingestion by young females results in significant increases in serum total testosterone concentration and other significant changes in the hormonal profile.
CHAPTER 1
INTRODUCTION

Testosterone enhances the development of muscle and strength. There are currently several nutritional supplements on the market advertised to either "increase the natural release" of testosterone, or "enhance its metabolic effects." Although oral supplementation of androstenedione has been suggested to increase blood testosterone concentrations, the conversion of androstenedione to testosterone in both males and females is a complex process and not well understood.

Both pre- and post-menopausal females may be on androgen therapy for increasing testosterone levels and thus the alleviation of androgen deficiency symptoms such as premature ovarian failure and post-menopausal and glucocorticosteroid-related bone loss (12). However, there is not valid research which closely examines the hormonal response to oral androstenedione ingestion in females. In addition, amateur and professional female athletes and competitors often are looking for a legal ergogenic aid to possibly improve their performance by the slightest edge. Discovering a supplement, such as androstenedione, which increases testosterone concentration with supplementation may be a beneficial ergogenic aid for such athletes and competitors.

Dehydroepiandrosterone (DHEA) and its sulfate ester (DHEA-s) are androgenic hormones produced primarily by the adrenal glands, which serve as immediate precursors to androstenedione (30). In the adrenal cortex, blood-borne DHEA can be converted to androstenedione, which later can be dehydrogenated in the liver to testosterone (30).
Dehydroepiandrosterone supplementation has been shown to increase testosterone concentrations in women (36,37,38,46).

Androstenedione is an immediate precursor to the formation of testosterone through the action of the 17 β-hydroxysteroid dehydrogenase enzyme. Androstenedione can also be aromatized to estrone through the action of the aromatase enzyme. However, the biosynthesis of testosterone from androstenedione and other androgens differs between the genders, which makes increasing testosterone levels from oral androstenedione supplementation in women unclear. In men, research has shown that a single ingestion of <300 mg androstenedione does not increase serum testosterone concentrations (3,8,25,27,41,49). However, in women approximately 60% of plasma testosterone is derived from plasma androstenedione, while only 2% of plasma testosterone results from plasma androstenedione in men (22). Since testosterone formation from androstenedione is greater in women and oral androstenedione has been shown to increase serum androstenedione concentrations in women (36,37,38,46), women may show increases in serum testosterone with androstenedione supplementation. Only one study has looked at the acute effects of androstenedione supplementation on serum testosterone concentrations in women (32). A 100-mg dose of androstenedione was ingested in this study, which resulted in significant increases of serum testosterone in two female subjects.

Since serum androstenedione is a quantitatively more important precursor to testosterone in females, we hypothesize that oral supplementation of androstenedione will increase serum testosterone concentration levels in women. Therefore, the purpose of this study is to examine the acute effects of 100- and 300-mg oral androstenedione on serum testosterone concentration and other sex steroid hormone concentrations.
CHAPTER 2

REVIEW OF LITERATURE

The use of supplements to increase testosterone and therefore enhance muscle growth raises skepticism in sports and society. Research suggests that oral administration of androstenedione of <300 mg does not increase testosterone in males (3,8,25,27,41,49), while a 300-mg dose produces a small and transient effect (27). The acute hormonal effects of orally administered androstenedione have been far less studied in females, with only one previous study conducted. The following review of literature will examine: (a) the enzyme kinetics of two major enzymes in the conversion of androstenedione to testosterone and estrogens; (b) ovarian steroidogenesis; (c) ovarian hormones; (d) androstenedione research in males; (e) androstenedione research in females; and (f) possible reasons for different hormonal responses to androstenedione between genders.

**Enzyme Kinetics**

It has been observed previously in young men that ingestion of androstenedione caused a significant increase in serum estradiol (8,25,27,41). In men, the enzyme kinetics for the aromatization of androstenedione to estrone ($K_m=25 \text{ nmol} \cdot \text{L}^{-1}$) (37) catalyzed by P450 aromatase favor the production of estrogens when compared to the 17ß-hydroxysteroid dehydrogenase conversion of androstenedione to testosterone ($K_m=1,500 \text{ nmol} \cdot \text{L}^{-1}$) (37). Thus, the differences in the Michaelis constant between the two major enzymes involved in testosterone and estrogen conversion support the findings of enhanced serum estradiol concentrations with androstenedione ingestion in men. In women, the Michaelis constant of both P450 aromatase and 17ß-hydroxysteroid dehydrogenase in the conversion of
androstenedione to estrone and testosterone, respectively, are currently unknown. However, it is thought that the difference between the two Michaelis constants is less in females and thus possibly tends to convert more androstenedione to testosterone compared to males.

Strict enzymatic regulation of testosterone ultimately derives from the conversion of both 4-androstene-3,17-dione (=4) and 5-androstene-3 β,17β-diol (=5diol) (45), while =4 oral (32) and percutaneous (15) supplementation has readily observed increases in in vivo testosterone concentrations in females. Therefore, hypotestosteronic individuals such as females appear to favor the conversion of androstenedione to testosterone through the enzymatic activity of 17β-hydroxysteroid dehydrogenase and therefore less aromatization of androstenedione to estrogens as compared to males.

**Ovarian Steroidogenesis**

The ovarian steroids include estrogens (estradiol, estriol, estrone), progestins (progesterone, 17β-hydroxyprogesterone), and androgens (dehydroepiandrosterone, androstenedione, testosterone). These ovarian steroids are produced by thecal and granulosa cells. The main steroids produced by the ovary are progesterone and estradiol (see Fig. 1). Although some androgens, particularly androstenedione and testosterone, are secreted into the bloodstream, a significant portion of androgens are converted to estradiol through the action of the aromatase enzyme complex in the ovary and therefore never released into the bloodstream as androstenedione or testosterone. In addition to androstenedione and testosterone, the ovary also secretes estrone, 17α-hydroxyprogesterone, 20α-hydroxyprogesterone, and 5α-reduced androgens such as 5α-dihydrotestosterone and 3α-androstenediol.
Figure 1. Biosynthesis of steroids in the ovary. 1, Cholesterol side-chain cleavage enzyme complex; 2, 3 β-hydroxysteroid dehydrogenase; 3, 17α-hydroxylase; 4, 17,20-lyase; 5, aromatase; 6, 17 β-hydroxysteroid dehydrogenase

Steroid Biosynthesis

The initial precursor for steroid biosynthesis is cholesterol (see Fig. 1), which comes from the plasma low-density lipoprotein (LDL)-associated pool, intracellular lipid droplets, and through de novo local synthesis (16). Cholesterol, which has 27 carbons, is converted to the 21 carbon compound pregnenolone in a reaction catalyzed by an enzyme complex known as cholesterol side-chain cleavage enzyme. The reaction occurs in the mitochondria and is a rate-limiting step in the steroid biosynthetic pathway. Pregnenolone is converted into progesterone by the action of a 3 β-hydroxysteroid dehydrogenase enzyme, or converted into
17 α-hydroxypregnenolone by a 17 α-hydroxylase. Progesterone and 17 α-hydroxypregnenolone can both be metabolized to 17 α-hydroxyprogesterone.

Further metabolism to androgens and estrogens results in further reduction in the number of carbons to 19 (androgens) and 18 (estrogens). 17 α-hydroxyprogesterone is the substrate for a 17,20-lyase enzyme that forms androstenedione (5,16,33). Androstenedione can also be synthesized from DHEA, a substrate for the enzyme 3 β-hydroxysteroid dehydrogenase. Androstenedione is either metabolized to estrone through the enzyme aromatase or converted into testosterone in a reaction catalyzed by the enzyme 17 β-hydroxysteroid dehydrogenase. Testosterone, like androstenedione, can also be metabolized into estradiol by the P450 aromatase enzyme (16,33). The biosynthesis of these ovarian steroids (estrogen, progesterone, androgen) occurs in interstitial granulosa cells and the cells of the follicular theca, where production is controlled by two main hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (16).

Control of Ovarian Steroid Production through LH and FSH

Ovarian steroids are produced under the control of LH and FSH. Only LH enhances the secretion of androgens. However, both LH and FSH stimulate the secretion of progesterone. The formation of estradiol also depends on both FSH and LH. Estrogen production, from androgens, is stimulated by LH, while the aromatase enzyme complex that catalyzes the conversion of androgens to estrogens is directly activated by FSH (see Fig. 2).
Figure 2. The control of ovarian estrogen, progesterone, and androgen production by LH and FSH. Cholesterol side-chain cleavage enzyme complex (SCC); 3β-hydroxysteroid dehydrogenase (3β-HSD); Luteinizing hormone (LH); Follicle-stimulating hormone (FSH).

Thecal and Granulosa Cells – The “Two-Cell” Hypothesis

The predominant site of androgen synthesis in the preovulatory follicle is the theca interna (thecal cell). Thecal cells can be compared to the Leydig cells of the testis in males. Although the thecal cells can produce estrogen, the predominant hormones are the androgens DHEA and androstenedione. Granulosa cells are the predominant site of estradiol production in the preovulatory follicle, and can be compared to the testicular Sertoli cells. Since thecal cells provide the precursors for granulosa cell estrogen production, both cell types are
Figure 3. Two-cell theory of early follicular steroidogenesis. Luteinizing hormone (LH) stimulates thecal steroidogenesis; primary product is androgen. Follicle-stimulating hormone (FSH) stimulates granulosa cell aromatization of thecal androgens to produce estrogen.

necessary for optimal estrogen production (23) (see Fig. 3).

Both thecal cells and granulosa cells, which produce progesterone and androgens, have LH receptors. The cells' exposure to LH results primarily in increased production of progesterone and a small production of androgens due to their limited 17,20-lyase activity (23). However, only granulosa cells have FSH receptors, which are the only ovarian cells with high aromatase activity.
Conclusion

The synthesis of ovarian steroids is a complicated enzyme-regulated process within follicles of the ovary, which begins with the conversion of cholesterol. Under the influence of LH, the thecal cell produces androgens that upon diffusion to the granulosa cell of the follicle are converted to estrogens through a FSH-supported aromatization reaction. This interaction between the thecal cell and granulosa cell compartments of the ovarian follicles, which is controlled by LH and FSH, helps explain how ovarian steroidogenesis is controlled.

Estrogens

Estrogens are predominant ovarian hormones that include 17β-estradiol (estradiol), estriol, and estrone. Both estrone and estradiol are present in the bloodstream. Estradiol is secreted into the bloodstream directly by the ovary. Estrone derives largely from peripheral conversion of estradiol or androstenedione, where it is further metabolized to estriol in the liver. Most circulating estrogens (~80%) are bound to proteins. Approximately 60% of the estrogen transported in the bloodstream is bound to a steroid-binding protein called sex hormone-binding globulin (SHBG), otherwise known as testosterone-binding globulin (TeBG), for which estrogens have a higher affinity (16,26). The remaining estrogen not in free form (~20%) is bound to albumin, for which estrogens have a low affinity (16,26,30). However, in comparison with androgens, estrogens have a lower affinity for SHBG, which results in a greater availability of estrogens to the tissues because only free steroids are transported into their target cells.

Estrogens are degraded in the liver to inactive metabolites, conjugated with sulfate or glucuronide, and excreted in the urine. Major metabolites of estradiol include estrone, estriol, and catecholestrogens (40).
Testosterone

Testosterone is a steroid hormone produced in the ovary and adrenal glands, while also being formed in peripheral tissues from androstenedione and to some extent from DHEA and $\Delta^5$-androstenediol. Testosterone is formed from cholesterol, which can be synthesized de novo or derived from low-density lipoproteins (16,28,50), while the secretion of testosterone is regulated by the hypothalamus through the release of LH. Testosterone is the primary physiologically active androgen in women, which acts on target tissues directly or after it is converted to 5 $\alpha$-dihydrotestosterone. Increased levels of testosterone are associated with increased strength and power (43,48).

As in men, ~98% of the total circulating testosterone in women is bound to SHBG, thus leaving only 2% of the total plasma testosterone (free testosterone) available for metabolism (5,16,33). However, SHBG is found in higher concentrations in the plasma of women compared to men (44). As a result, the percentage of testosterone bound differs in women (96%) as opposed to men (93%).

Excretion of testosterone and its metabolites occurs primarily in the urine. Androstenedione and etiocholanolone account for approximately 50% of the daily turnover of testosterone metabolites, while the other half is in the form of inactive polar metabolites (diols, triols, and conjugates) formed in peripheral tissue (16). Similar to estrogens, testosterone and its metabolites are excreted mostly in the urine.

DHEA

Dehydroepiandrosterone, and its major precursor DHEA-S, are hormonal precursors to testosterone, estrone, and estradiol. Dehydroepiandrosterone is converted in peripheral tissues to androstenedione, testosterone, and dehydrotestosterone (DHT) (2,6), and is also
aromatized to estrone and estradiol (30). Although the biosynthesis of DHEA and DHEA-S from cholesterol seem to be accomplished through pathways identical to those in male testis, there are gender differences in the production of such androgens in relation to testosterone formation.

**Gender Differences**

Dehydroepiandrosterone is converted in tissues to testosterone by a greater percentage in females compared to males (2,6,21). Haning et al. (18,19) suggested that circulating DHEA-S in females is taken up by the ovarian follicle and becomes an important source of testosterone within the follicle that is excreted into the blood. Therefore, DHEA-S serves as an important pre-hormone for circulating testosterone in women. Haning et al. further suggested that DHEA-S is also an important precursor of ovarian testosterone after the administration of menotropins (17) and can be increased under the influence of FSH. Bird et al. (6) observed that women have a greater percentage of conversion of DHEA to testosterone than men, with reported values of 0.9% and 0.6%, respectively. Since androstenedione is converted to DHEA in a reaction catalyzed by 3 β-hydroxysteroid dehydrogenase, androstenedione supplementation could theoretically increase DHEA levels that result in a greater amount of testosterone being converted in females. Therefore, androstenedione supplementation could increase testosterone in females to a higher concentration compared to males.

**Supplementation**

Ingestion of DHEA does not acutely affect testosterone concentrations in males (7). However, compared to males, female research suggests different results (36,37,38,46). Morales et al. (37) observed increases in serum testosterone and DHT in postmenopausal
women after 6 months of a 100-mg daily dose of DHEA supplementation. Supplementing half of the dosage (50-mg) of DHEA daily for six months, Morales et al. (36) observed two-fold increases in serum testosterone and DHT in postmenopausal women. The majority of past research relating DHEA supplementation with testosterone concentrations has been done on postmenopausal women. It is unclear how premenopausal females would respond to DHEA supplementation as compared to postmenopausal females. Due to the differences in LH and FSH cycle patterns and enzymatic activities of converting androgens to estrogens, perhaps premenopausal may also benefit in testosterone concentrations following DHEA supplementation. Therefore, DHEA supplementation in premenopausal women demands further research.

Although both DHEA and DHEA-S are aromatized to estrogens in non-pregnant women, the amount of estrogens formed is too small to be an important source of circulating estrogens in young women (19,31). Longcope et al. (29) observed an increase in the aromatization of DHEA to estrogens in postmenopausal women compared with college-aged women. Therefore, less aromatization of DHEA to estrogens in college-age women could provide the basis for greater conversion to testosterone and thus higher testosterone levels.

Females have higher DHEA to testosterone conversion percentages than males. Premenopausal females aromatize less estrogen and thus allow more DHEA to be converted to androgens than postmenopausal females. Since DHEA and androstenedione are interconvertable, androstenedione supplementation could substantially increase testosterone concentrations especially in premenopausal females.

**Androstenedione**
Androstenedione is an important source of plasma testosterone in women. Both the stroma cells of the ovaries and the adrenal glands secrete androstenedione. The majority of androstenedione formed is converted by the aromatase enzyme to estrone in the liver, in a similar manner to which testosterone is aromatized to estradiol (16,33). However, some androstenedione is converted to testosterone through the action of the 17 β-hydroxysteroid dehydrogenase enzyme before entering the blood plasma (5,33).

Gender Differences

In contrast to testosterone, plasma androstenedione concentrations are higher in women than in men (20,22). A significant amount of testosterone in women is derived from blood androstenedione. Horton and Tait (22) observed that nearly 60% of plasma testosterone in women is derived from plasma androstenedione by peripheral conversion compared with less than 2% in males. The production rate or secretion of androstenedione was also found to be higher in women, 3.4 mg/day compared to the 1.4 mg/day in males (22). These authors (22) also observed that 6% of the orally administered androstenedione enters the general circulation as plasma androstenedione, while ~2% enters the plasma as testosterone in women.

Androstenedione appears to be the major androgen secreted in young adult females since it gives rise to most of the circulating plasma testosterone. The production of androstenedione rather than testosterone in women but not in men is a major difference in androgen synthesis between genders. The function of testosterone and DHT derived from other blood precursors is also different between genders. In men, nearly all plasma testosterone results from direct testicular secretion of testosterone. Ito and Horton (24) observed that of the 253 µg of DHT produced per day in men, 203 µg (59%) was derived
from testosterone and 50 µg (14%) from androstenedione. The production of DHT in women
(41 µg/day) primarily reflects the conversion of 36 µg of androstenedione and 3 µg of
testosterone into DHT, suggesting an absence of direct secretion of DHT (24,47).

There are various differences between genders in regard to androstenedione. A major
difference which may explain why females respond differently to androstenedione
supplementation is that 60% of plasma testosterone is derived from plasma androstenedione
in females, in contrast to only 2% of plasma testosterone in males. Other major differences
with females compared to males include that androstenedione is a more important source of
plasma testosterone, a greater proportion of testosterone is derived from androstenedione,
and androstenedione is the major secreted androgen giving rise to most of the circulating
plasma testosterone.

Supplementation

The acute effects of oral androstenedione administration on serum testosterone
concentrations in men are not well established. Two recent studies concluded that serum
testosterone concentrations in men increase after ingestion of androstenedione (13,27).
Earnest et al. (13) reported that the incremental area under the curve for serum testosterone
concentrations during 90 minutes following the ingestion of 200-mg androstenedione was
higher compared with placebo. Following ingestion, however, total testosterone and free
testosterone were not significantly higher at any time point. The authors reported the values
for area under the curve by apparently including the area attributable to baseline serum
testosterone concentrations, which were slightly higher in the androstenedione-supplemented
group of subjects.
King et al. (25) were the first researchers to show that a 100-mg dose of oral androstenedione supplementation does not increase serum testosterone concentrations in young men. A single 100-mg dose given to ten normotestosterogenic men did not increase serum testosterone for up to six hours. The authors also observed a lack of enhancement in skeletal muscle adaptation to resistance training with androstenedione supplementation over an 8-week period, when given three 100-mg doses daily.

In agreement with King et al. (25), later research agreed with these findings (3,8,27,41,49). Leder et al. (27) observed no change in serum testosterone concentrations for 8 h after subjects ingested 100-mg androstenedione, in agreement with previous acute response research with identical dosage (3,8,25,41,49). Leder et al. (27) also found no change in basal serum testosterone concentrations during one week of 100- or 300-mg single daily doses of androstenedione. However, the authors observed a mean increase in serum testosterone concentrations by ~34% during 8 h following a single 300-mg dose of androstenedione. These results suggest single doses of <300-mg of androstenedione do not increase serum testosterone in young men, while 300-mg produces a small and transient increase.

The acute effects of androstenedione supplementation in women are unclear and are far less studied. The only research study testing the effect of androstenedione supplementation on serum testosterone concentrations in women observed substantial elevations in testosterone concentrations of two healthy women (32). In these women, 100-mg of ingested androstenedione resulted in increases in serum androstenedione concentrations from 0 to 5 nmol/L and increases of total testosterone concentrations from 3
to 18 nmol/L. The authors failed to describe the two subjects in terms of age or reproductive status.

**Conclusion**

Some of the underlying mechanisms for possible differences in serum testosterone responses which have been mentioned previously include: 1) higher percentage conversion of DHEA to testosterone in women; 2) higher TeBG concentrations in women; 3) higher amounts of plasma testosterone derived from plasma androstenedione in women; 4) higher overall plasma androstenedione concentrations in women; and 5) higher secretion rate of androstenedione in women. Since only one poorly designed and executed study has been conducted on the effects of oral androstenedione ingestion in females, the extent to which oral androstenedione affects sex steroid concentrations in women should be determined.
Subjects

Eight healthy college-aged females were recruited for this experiment. Before participating in this study, subjects signed an informed consent form previously approved by the Iowa State University Human Subjects Review Committee. Subjects completed a self-administered written medical history to eliminate any subjects with known metabolic disorders or abnormal menstrual cycles. All subjects were screened to ensure no oral contraceptive use. In addition, all subjects were screened regarding previous or current use of nutritional supplements and to ensure no current use of androstenedione.

Design of Study

On three separate occasions, separated by one month and after an overnight fast, a 100-mg androstenedione, 300-mg androstenedione, or placebo (83-mg rice flour) was administered in a randomly assigned double-blind manner. Dietary intake was assessed for three days prior to testing, with this same dietary pattern replicated by each subject before each testing period. Blood samples were obtained before and every thirty minutes post ingestion up to 240 minutes (4 h). Serum hormone concentrations were determined as described below.

Experimental Procedures

Subjects reported to the laboratory following a 10-12 hour overnight fast between 0600 and 0900, during the follicular phase of the menstrual cycle (day 3-5 after menstruation). Prior to supplementation, a baseline blood sample (0 min) was taken. Before
each 10-ml blood sample was obtained for serum hormonal analysis, a 2-3 ml blood sample was obtained first to ensure a "clean" sample for analysis. Blood samples were drawn with a 12-ml syringe from a flexible teflon catheter inserted into an antecubital vein. Blood was immediately transferred to a serum collection tube containing SST gel and clot activator. After clotting, the blood was placed on ice until centrifugation. After centrifugation the serum was pipetted off and frozen at −80° C until assayed for hormone assessment as described below.

**Diet Record**

Dietary intake was replicated for three days prior to each test day. Subjects recorded a 3-day diet record prior to the morning of trial 1, with a similar dietary pattern repeated for the remaining two trials. Subjects were queried frequently and reminded to ensure that they maintained a semi-controlled diet. A dietary analysis was run on each subject’s diet (Nutritionist IV).

**Anthroprometric Data**

Height was measured prior to trial one and weight was measured prior to each trial using a Deteco™ (Webb City, MO, USA) upright wall stadiometer with .5 cm and .1 kg precision, respectively, to monitor weight fluctuations. Body composition was determined between trials 2 and 3 using hydrostatic weight. Eight trials of underwater weighing were performed per subject, while the lowest two identical body masses in water were used for body density calculation. Body density and body fat percentage were calculated using the techniques specified and Broyile formulas given by the American College of Sports Medicine (1).
Hormonal Analyses

Serum concentrations of androstenedione and total testosterone were measured with radioimmunoassay (RIA) using commercially available kits (Diagnostic Products, Los Angeles, CA). Serum concentrations of estradiol were measured with RIA using commercially available kits (Diagnostic Systems Laboratories, Webster, TX).

Statistical Analysis

Data were analyzed using commercial software (SPSS Inc, Chicago, Ill). A statistical analysis was performed using two-way (time and treatment) analyses of variance (ANOVA) with repeated measures. When ANOVA revealed a significant interaction (P<0.05), specific mean differences were located with a Newman-Keuls multiple-comparison test.
CHAPTER 4

RESULTS

Subjects

Of the ten subjects who started this project, two subjects withdrew due to time conflicts and menstrual cycle problems.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age (y)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Body Fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 8</td>
<td>22.1 ± 0.4</td>
<td>165.0 ± 3.8</td>
<td>58.6 ± 4.2</td>
<td>20.3 ± 2.0</td>
</tr>
</tbody>
</table>

Values are means ± SE.

Table 1. Age and anthropometric data of the subjects.

Serum Androstenedione Response

The baseline serum androstenedione concentration (Figure 4) was not different in placebo (PLA), the 100-mg dose (LO), or the 300-mg dose (HI) prior to ingestion. Serum androstenedione concentrations increased 60 min after ingestion in both LO and HI and remained elevated compared with PLA during the 240 min after ingestion (p < 0.05). The serum androstenedione concentration was higher (p < 0.05) in HI compared with LO from 120 to 240 min. At 240 min, serum androstenedione concentrations in LO (22.6 ± 1.0 nmol/L) and HI (28.1 ± 1.1 nmol/L) were 317% and 410% higher, respectively, compared with PLA (5.5 ± 0.8 nmol/L). The area under the curve (AUC) for LO and HI (Figure 5) was higher than PLA (p < 0.05) and was higher in HI (3151.9 ± 380.0 nmol/L•min) compared with LO (2245.1 ± 346.5 nmol/L•min).
Figure 4. Serum androstenedione response to intake of placebo (PLA), 100-mg (LO), or 300-mg (HI) androstenedione.

* LO and HI significantly different from PLA, $p < 0.05$
† HI significantly different from LO, $p < 0.05$

Figure 5. Area under the curve (AUC) of serum androstenedione response to intake of placebo (PLA), 100-mg (LO), or 300-mg (HI) androstenedione.

* PLA significantly lower than LO and HI; † HI significantly higher than LO, $p < 0.05$
Serum Estradiol Response

Serum concentrations of estradiol (Figure 6) were not different prior to androstenedione ingestion before 150 min in PLA, LO, or HI. Serum concentrations of estradiol was higher in LO compared with PLA at 150 min (p < 0.05). Serum concentrations of estradiol were higher at each time point throughout min 150 to 240 in HI compared with PLA (p < 0.05). A 62% increase in serum estradiol concentration at 210 min was observed in HI above that of PLA. The estradiol serum concentration was not different in LO or HI at any time point.

Figure 6. Serum estradiol response to intake of placebo (PLA), 100-mg (LO), or 300-mg (HI) androstenedione.
* LO significantly different from PLA, p < 0.05
† HI significantly different from PLA, p < 0.05
Serum Total Testosterone Response

Serum total testosterone concentration (Figure 7) was not different in PLA, LO, or HI at baseline through 60 min after ingestion. Serum total testosterone concentrations in HI and LO were higher (p < 0.05) compared with PLA at 60 min and 120 min, respectively, and remained higher through 240 min. At the 210 and 240 min point, serum total testosterone concentrations in both HI (10.2 ± 1.6 nmol/L) and LO (5.5 ± 0.9 nmol/L) were ~627% and ~293% higher, respectively, compared with PLA (1.4 ± 0.2). There was no difference in serum total testosterone concentration between LO and HI before 120 minutes. Serum total testosterone concentration was higher (p < 0.05) in HI compared with LO from 120-240 min, with an 85% higher serum total testosterone concentration in HI at 240 min after ingestion. The AUC was higher in HI (1343.3 ± 257.8 nmol/L·min) compared with LO (631.9 ± 94.2 nmol/L·min; p < 0.05).

Figure 7. Serum total testosterone response to intake of placebo (PLA), 100-mg (LO), or 300-mg (HI) androstenedione.
* Significantly different from PLA; † HI significantly different from LO, p < 0.05
Figure 8. Area under the curve (AUC) of serum total testosterone concentration response to intake of placebo (PLA), 100-mg (LO), or 300-mg (HI) androstenedione.

* PLA significantly different from LO and HI, p < 0.05
† HI significantly different from LO, p < 0.05
CHAPTER 5
DISCUSSION

A major finding of this study is that short-term androstenedione supplementation increased the serum total testosterone concentration in young women with normal serum testosterone levels. Furthermore, serum total testosterone was higher after ingestion of 300-mg androstenedione compared to 100-mg.

The only prior report on androstenedione administration in women (32) also demonstrated substantial elevations in the blood testosterone concentration in two healthy women, but to a greater extent than the increase seen in this present study. Mahesh and Greenblatt (32) showed that 100-mg of androstenedione increased blood total testosterone from 3 to 18 nmol/L (300%) 60 min after ingestion, with a plateau at 90 min. In the present study, 100-mg of androstenedione increased serum total testosterone concentration from 1.2 to 5.5 nmol/L at 240 min after ingestion. Serum total testosterone concentration was only increased by 2 nmol/L from baseline after 60 min, in contrast to the 15 nmol/L increase at 60 min after intake seen by Mahesh and Greenblatt (32) with a 100-mg dose. Ingestion of 300-mg androstenedione in the present study led to only an 8 nmol/L increase in serum total testosterone concentration after 240 min. The prolonged serum testosterone response, along with the milder increase in serum testosterone seen in the present study, leads to speculation of possible but unknown differences between the Mahesh and Greenblatt (32) and the present study.

The response of serum androstenedione concentration after ingestion in the present study contrasts with Mahesh and Greenblatt (32) and may help explain the lower increase in
serum total testosterone seen in this present study. Mahesh and Greenblatt (32) showed that 100-mg of androstenedione increased the blood androstenedione concentration from 0 to 5 nmol/L at 60 min after ingestion. In the present study with 100-mg androstenedione, a 6 to 11 nmol/L increase in serum androstenedione concentration after 60 min was seen with a continuing elevation to 23 nmol/L after 240 min. The higher serum androstenedione response observed in the present study, associated with a lower total testosterone response, may indicate less conversion to serum total testosterone through 17 β-hydroxyysteroid dehydrogenase.

Age differences in the subjects between the present study and Mahesh and Greenblatt (32) may also explain the higher increase in testosterone concentration seen in Mahesh and Greenblatt (32). The unknown ages of the two subjects in Mahesh and Greenblatt, along with a 15 nmol/L higher blood total testosterone concentration at 60 min after ingestion, leads to speculation that the subjects were postmenopausal. Postmenopausal females with hypoestrogenic conditions may respond with a higher blood total testosterone concentration after androstenedione supplementation (38, 46). Despite not assaying for estrone or measuring the enzymatic kinetics of P450 Aromatase and 17 β-hydroxyysteroid dehydrogenase in the present study, it is the author's opinion that the lower aromatizing activity of androstenedione to estrogens through P450 aromatase in postmenopausal women compared to normoestrogenic premenopausal women may result in higher conversion rates of androstenedione to testosterone through the enzymatic activity of 17 β-hydroxyysteroid dehydrogenase. In addition, the possible use of postmenopausal subjects, along with an unknown hormonal status and day of menstrual cycle during the testing periods employed by Mahesh and Greenblatt (32), may result in contrasting conversion rates between androgens
and estrogens and thus possibly explain a greater increase in total testosterone concentration compared with what we are reporting in the present study.

The time period in which testosterone concentration returned to baseline levels after androstenedione ingestion is in contrast to findings from Mahesh and Greenblatt (32). The present study obtained blood samples for 240 min after ingestion, in contrast to 90 min by the Mahesh and Greenblatt (32) study. Previously, 100-mg of androstenedione administration in men has shown that blood androstenedione concentrations level off approximately 4 h after ingestion (25,27,41). A longer sustained elevation in blood androstenedione concentration seen in past research in males (25,27,41) and in this present study is in contrast to Mahesh and Greenblatt (32), demonstrating that blood androstenedione concentration plateaued at 60 min and dropped at 90 min after intake. The reason for a shorter testosterone response observed by Mahesh and Greenblatt (32) compared to the more prolonged testosterone response seen in this present study and past research in men is unknown.

The increased total testosterone concentration after androstenedione ingestion seen in this present study and previous research in females (32) is in contrast to previous research in males (3,8,25,27,41,49). For example, King et. al. (25) showed higher relative increases in estradiol compared to this present study, where 100-mg only increased estradiol at 150 min and 300-mg increased estradiol from 150-240 min. It may be possible that following the androstenedione ingestion in females, more of the ingested androstenedione remained as estrone rather than being converted further to estradiol. King et. al. (25) suggested that the unchanged serum testosterone concentration following androstenedione ingestion, in spite of the higher androstenedione concentration, is due to an increased formation of estrogens from the exogenous androstenedione. The smaller relative increase in estradiol seen in the present
study compared with research in males (3,8,25,27,41,49) suggests that a significant proportion of the ingested androstenedione was preferably converted to testosterone through the enzymatic activity of 17 β-hydroxysteroid dehydrogenase, with less androstenedione undergoing aromatization to estrogens. A purported preferred conversion of ingested androstenedione to testosterone in females is reasonable since 6% of the orally administered androstenedione enters the general circulation as plasma androstenedione (22). In addition, nearly 60% of plasma testosterone is derived from plasma androstenedione by peripheral conversion (22). The 60% of plasma testosterone derived from androstenedione in females is far greater than the 2% of plasma testosterone derived from androstenedione in men (22). Therefore, less estrogen conversion from ingested androstenedione, along with a higher percent conversion of plasma testosterone from androstenedione, implicates a possibly higher testosterone response to androstenedione ingestion in women compared with men.

Androstenedione is converted to DHEA in a reaction catalyzed by 3 β-hydroxysteroid dehydrogenase, while DHEA is further converted to testosterone. The percentage of DHEA conversion back to androstenedione and furthermore to testosterone in tissues is greater in females compared to males (2,6,21). Previous research on DHEA supplementation has shown an increase in testosterone concentration in females (36,37,38,46), while it is unchanged in males (7). In addition, circulating DHEA-S in females is a more important precursor for testosterone, since DHEA-S is taken up by the ovarian follicle (18,19). Therefore, a greater DHEA-S concentration coming from the conversion of androstenedione may result in a higher testosterone concentration in females compared with males. Although DHEA concentration was not measured in this study, it is possible that DHEA concentrations were
increased from the ingested androstenedione and thus further contributed to the higher testosterone response seen in females (32) compared to males (3,8,25,27,41,49).

An increase in testosterone as a result of androstenedione supplementation may be desirable for amateur and professional female athletes. Testosterone stimulates muscle protein synthesis by activating a hormone receptor complex that increases mRNA and DNA synthesis (11,16,22). Therefore, a higher testosterone concentration may be beneficial for female athletes and competitors seeking an increase in muscle mass and muscular strength (11). In addition, androgen therapy is becoming more popular in not only postmenopausal females experiencing postmenopausal and glucocorticosteroid-related bone loss, but also premenopausal females who show androgen deficiency symptoms such as premature ovarian failure.

Although their appear to be potential benefits to androstenedione supplementation in females, an increase in testosterone concentration may result in potentially serious adverse health consequences in young females. Above normal testosterone concentrations in females may be associated with hirsutism (34), especially when the testosterone/SHBG ratio increases (9). High testosterone concentrations in females also increase a number of the risk factors for atherosclerotic cardiovascular disease. A 9-fold increase in serum testosterone has been observed to increase the integrated insulin concentration without a change in fasting glucose insulin values (38). This adverse affect on insulin sensitivity due to increased testosterone concentrations may contribute to diabetes and/or an increased risk of renal injury (42). In addition, a drop in HDL-cholesterol values is associated with increased testosterone concentrations in both males and females (4,10,35,38). For example, Barnkart et. al. (4) showed a 10% decrease in HDL-cholesterol concentrations accompanied by a 95% increase
in testosterone concentration. Finally, an increase in hypertension (42) and impaired flow-mediated dilation and vascular reactivity (35) have been associated with above normal increased testosterone concentrations in females.

In summary, acute administration of 100- and 300-mg androstenedione significantly increased the blood total testosterone concentration in healthy college-aged women, but significantly less than the only research previously conducted in female subjects. However, the ability to increase serum testosterone concentrations following orally ingested androstenedione is in contrast with research in males showing no effect. A lower estrogen response, along with gender differences in the conversion rates of androstenedione in forming testosterone, provides a possible explanation for a different testosterone response between genders. Although an increase in testosterone may be desirable in some populations (i.e., pre- and postmenopausal females with androgen deficiency symptoms), the potential health consequences and benefits from increased testosterone concentrations should be considered when conducting further research in females using androstenedione supplementation.
REFERENCES CITED


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