Androgenic supplementation in men: effects of age, herbal extracts, and mode of delivery

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Androgenic supplementation in men: Effects of age, herbal extracts, and mode of delivery

by

Gregory Allen Brown

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CHAPTER 1. INTRODUCTION

Androgenic hormones, such as dehydroepiandrosterone (DHEA), androstenedione, and androstenediol, are sold as nutritional supplements for the purposes of increasing serum testosterone concentrations and enhancing the adaptations to resistance training. Use and sales of androgenic hormone supplements dramatically increased following the highly publicized use of androstenedione by Mark McGwire during the 1998 Major League Baseball season when he set a new single season home run record (1,2).

The purported efficacy of androstenedione as an anabolic agent is based upon a single study in which 100 mg androstenedione intake in two women increased serum testosterone concentrations ~ 6 fold (3) and a patent application from Germany that claimed remarkable increases in serum testosterone following androstenedione intake (4). Despite the popularity of androstenedione as a nutritional supplement, there was no evidence that androstenedione was an anabolic agent.

In 1999, King et al. (5) were the first to report that ingesting 100 mg androstenedione t.i.d. day does not increase serum testosterone concentrations, muscle mass, or strength in young men. Other researchers also reported that ingesting 100 mg androstenedione does not increase serum testosterone concentrations (6-8) or increase muscle protein synthesis (8) in young men.

Beginning around age 30, there is a steady decline in serum testosterone concentrations as men age (9). Since androstenedione ingestion increases serum testosterone concentrations in women (3), who have much lower testosterone concentrations than young men, ingesting testosterone precursors may increase serum testosterone concentrations in
older men. Therefore, one purpose of this project was to determine the serum testosterone response to ingesting 100 mg androstenedione or androstenediol t.i.d. in men 30 – 60 years old.

While testosterone precursor ingestion has no effect on serum testosterone concentrations, ingesting androstenedione or androstenediol does increase serum estrogen concentrations (5-8,10). Indole-3-carbinol (I3C) and chrysin are herbal extracts reported to prevent the aromatization of androgens into estrogens (11-14). Hence, ingesting I3C and chrysin in conjunction with testosterone precursors may prevent the conversion of the testosterone precursors into estrogens.

Androstenedione and androstenediol can also be converted to dihydrotestosterone (DHT) (15,16). Saw palmetto and gamma linolenic acid (yla) are herbal extracts that may inhibit the enzyme $5\alpha$ reductase that converts androgens into DHT (17-20). Thus, ingesting saw palmetto and yla may prevent the conversion of testosterone precursors into DHT.

Tribulus terrestris (TT) is an herbal extract claimed to enhance serum luteinizing hormone concentrations with a subsequent increase in serum testosterone, although this has not been proven.

Hence, the use of chrysin, I3C, saw palmetto, yla, and TT in conjunction with testosterone precursors may prevent the conversion of testosterone precursors into estrogens or DHT, resulting in increased serum testosterone concentrations. Although ingesting a nutritional supplement that contains androstenedione, DHEA, saw palmetto, TT, I3C, and chrysin does not increase serum testosterone concentrations in young men (21), the effects of a nutritional supplement that combines herbal extracts with testosterone precursors in older
men is unknown. Another purpose of this project was to determine the hormonal response to testosterone precursor nutritional supplements that include herbal extracts in older men.

Ingested androgens are subjected to a digestive catabolism and hepatic extraction, limiting the amount of testosterone precursor that can enter the circulation and be converted to testosterone (22,23). The hormonal response to a testosterone precursor administered in a manner that bypasses digestion and first pass catabolism is currently unknown. The final purpose of this project was to determine the acute hormonal response to a mode of androgen delivery that bypasses digestion and first pass catabolism.

Dissertation Organization

Following this introduction and a review of literature, four manuscripts addressing the effects of androgenic hormone supplementation in men will be presented as separate chapters.


The final chapter discusses the conclusions that can be drawn from this body of work, and future directions for research. Since chapters 3-6 are previously published works, the figures will be referred to numerically as they are in the original publications and not sequentially by order of appearance in this dissertation.
CHAPTER 2. REVIEW OF LITERATURE

Introduction

Steroid biosynthesis is a complex process beginning with cholesterol, either from dietary sources or de novo synthesis, and ending with urinary excretion (Figure 1). Steroid hormones are formed in the adrenal glands, testes, and ovaries. In addition,

![Diagram of steroid biosynthesis and interconversion](image)

there is a great deal of hormonal interconversion that occurs within adipose tissue, muscle tissue, and other peripheral tissues (15,16,24,25). This review of literature will explore the
formation and interconversion of steroid hormones, the biological effects of these hormones, and the current state of knowledge regarding the use of androgens as nutritional supplements.

**Sex Steroids**

Sex steroids are cholesterol-derived hormones that influence the development of primary and secondary sex characteristics. Androgenic hormones are C-19 steroids that contribute to the development of male reproductive function. In addition to their role in the development of sexual characteristics, some androgenic hormones also have muscle building, or anabolic, properties (15,26,27).

Estrogenic hormones are C-18 steroids that have effects not only on feminine breast development, but also on bone growth, vascular structure and function, mental development, and reproductive function in both genders. Estrogens may counteract or accentuate the effects of androgens, depending upon the target tissue (28-31).

The formation of steroids from cholesterol is a complex process that involves multiple enzymatic steps and intermediary hormones. Once formed, androgens are converted into estrogens, DHT, or inactive metabolites in the peripheral tissues (Figure 1). Steroids are eliminated from the body in the form of inactive, glucuronidated metabolites and active urinary metabolites such as DHT and estriol (15).

**Testosterone**

Testosterone (17β-hydroxyandrost-4-en-3-one) is the principal androgen, formed primarily in the ovaries in females and testes in males. The secretion of testosterone is regulated by the hypothalamus through the release of luteinizing hormone (LH).

Testosterone is formed from cholesterol, which is synthesized de novo or derived from low-
density lipoproteins. Testosterone is also formed from precursor steroids such as androstenedione in peripheral tissues.

Testosterone has both androgenic and anabolic properties. Normal serum testosterone concentrations are ~10-20 fold higher in men than those found in women, which accounts for the larger muscle mass and strength commonly observed in males (15,32). Only testosterone free from sex-hormone-binding globulin (SHBG), representing ~2% of the total plasma testosterone (free, or bio-available, testosterone), is available for metabolism. Of the testosterone not available for metabolism, ~60% is bound to SHBG, and the remaining ~38% is bound to albumin (15,33-35).

Roughly one half of the daily excretion of testosterone metabolites is in the form of urinary androstenedione and etiocholanolone. The remaining urinary steroids are in the form of polar metabolites (diols, triols, and conjugates) formed from testosterone as a prohormone. These urinary metabolites are predominantly inactive (15,22).

Testosterone can be irreversibly converted to active metabolites such as DHT and estrogens (15,24,25,36,37). Dihydrotestosterone has no apparent effect on skeletal muscle (38) but has a higher binding affinity for both the androgen receptor and SHBG than does testosterone, making DHT a potent mediator of testosterone activity (35,39). Dihydrotestosterone, which is a 5α reduced steroid, has been linked to prostatic hypertrophy (40-43). In males, the majority of estrogens are formed by aromatization of androgens in the central nervous system and peripheral tissue (15,24,37). Estradiol formation involves sequential hydroxylation, oxidation, removal of the C-19 carbon, and aromatization of the A ring of testosterone. Of the 45 μg of estrogens produced each day by the average young man, 85-90% is from aromatization with the rest being directly formed in peripheral tissue (15,44).
Aging and Testosterone. In men, serum total testosterone concentrations remain constant throughout much of adulthood, and do not decline until the 7th or 8th decade of life. However, serum free testosterone concentrations peak in the third decade of life, and then gradually decrease throughout the remaining years due primarily to an increase in SHBG concentrations (9,27,45,46). This age-related decline in serum free testosterone concentration is thought to contribute to the reduced muscle mass, loss of libido, and increased adiposity in aged men (27,47).

Testosterone and Muscle Mass. Testosterone stimulates muscle protein synthesis by activating a nuclear hormone receptor complex that increases mRNA and DNA synthesis (15,48,49). Plasma testosterone concentrations increase transiently due to a bout of resistance exercise (50-53). Although resistance exercise does not alter serum testosterone concentrations beyond a transient increase (33,54,55), resistance exercise promotes increased muscle mass indicating that this brief elevation in serum testosterone has an effect on muscle protein synthesis.

Testosterone Administration. Anabolic steroids are exogenous substances taken either orally or intramuscularly to mimic or enhance the effects of testosterone on muscle protein synthesis. Early research indicated that anabolic steroids may not increase muscle mass or strength (56-58). However, many of these early studies used insufficient doses to cause an anabolic effect (56,59-61). Bhasin et al. (62) were the first to demonstrate conclusively that, when given in sufficient doses, anabolic steroids result in increased muscle mass and strength. Although the use of anabolic steroids is banned in many sports and the use of anabolic steroids is associated with negative health consequences (61,63), anabolic steroids
have an important therapeutic role in the treatment of hypogonadism (64-66), age-related androgen deficiency (67), and muscle wasting diseases (68).

**Mode of Delivery.** Oral ingestion of testosterone produces minimal effects on muscle mass and serum testosterone concentrations, due to the processes of digestive breakdown and hepatic catabolism. Horton and Tait (23) observed that as much as 89% of an ingested androgen is destroyed by the digestive process. Of the remaining androgen that reaches the blood, ~98% is destroyed by hepatic catabolism. Thus, very little of an ingested androgen reaches the target tissues.

Therefore, effective testosterone administration requires administration in a route that avoids digestion and hepatic extraction. One such method is sublingual administration, with the androgen being absorbed by the oral submucosa. Unfortunately, androgens are hydrophobic molecules that are not effectively absorbed across the submucosal membranes. However, the combination of testosterone with cyclodextrins, a starch-derived molecule that forms a hydrophilic inclusion complex with an androgen, increases in serum testosterone concentrations (64,65,69-71). Intramuscular injection of testosterone enanthate is another method of delivery that avoids digestion and hepatic catabolism and is frequently used for testosterone replacement therapy and as an anabolic agent (48,62,67). Another successful mode of androgen administration is ingested synthetic testosterone analogs (such as methandienone, stanozolol, and nandrolone) that are not destroyed by digestion and hepatic catabolism. However, these substances have more pronounced side effects than do other mode of testosterone administration (57,72,73).

**Side Effects with Anabolic Steroid Use.** Abnormally high concentrations of serum testosterone are associated with abnormal cholesterol metabolism (74,75). The use of
anabolic steroids is associated with a decrease in serum high-density lipoprotein cholesterol concentrations (HDL-C) (76,77) and specifically HDL-2, due to an increase of hepatic triglyceride lipase activity (78). The reduced HDL-C associated with anabolic steroid use is reversed once steroid use has been discontinued (68,79).

Use of exogenous testosterone and anabolic steroids is associated with impaired gonadal function due to negative hypothalamic-pituitary-adrenal feedback as evidenced by reduced serum LH and follicle stimulating hormone (FSH) concentrations. This impaired gonadal function results in lowered production of endogenous testosterone and impaired reproductive function (68,79). Although it has generally been thought that gonadal function returns after steroid use has been discontinued, it now appears that prolonged use of anabolic steroids may result in permanent hypogonadism (80).

Other negative side effects associated with anabolic steroid use include masculinization of women, gynecomastia in men, psychological dependency, aggression, baldness, and prostate enlargement (68,79). These side effect are not all directly related to testosterone, but are caused by the conversion of testosterone to DHT or estradiol.

**Dehydroepiandrosterone**

Dehydroepiandrosterone (3β-hydroxy-5-androsten-17-one) is formed from 17α hydroxypregnenolone via 17, 20 lyase primarily in the adrenal glands. Dehydroepiandrosterone and its sulfate ester, DHEA-S, are the most abundant steroids in the circulation. Serum concentrations of DHEA peak in the third decade of life and then progressively decline with advancing age (15,81). In spite of the large quantities of DHEA in the blood, its physiologic role is unclear.
Once formed, DHEA is converted to androstenedione via 3β hydroxysteroid dehydrogenase (3β HSD) (40,82) or androstenediol via 17β hydroxysteroid dehydrogenase (17β HSD) (83). Dehydroepiandrosterone is also converted to estrogens or inactive metabolites (15,40). It appears that the primary fate of endogenous DHEA is conversion to other hormones (84,85), with DHEA being an estrogen precursor in men and an androgen precursor in women (83,86).

**DHEA Intake.** The use of DHEA as a nutritional supplement is advertised to enhance feelings of health and vitality, provide anti aging benefits, as well as reduce obesity and improves athletic performance. However, research on DHEA intake has produced mixed results.

Ingesting DHEA increases serum DHEA and androstenedione concentrations in men and women alike (87-92). Ingesting DHEA also increases serum testosterone and DHT concentrations in women (89-91,93) but not in men (87-92). Ingesting DHEA also does not alter serum estrogen concentrations in men (88-92) except those over age 70 (87). The lack of change in serum testosterone or estrogens in spite of increased serum DHEA and androstenedione with DHEA ingestion is difficult to explain.

Labrie et al. (94) found that DHEA administration results in the formation of only inactive steroid metabolites in serum. They suggested that the inactive steroid metabolites formed with DHEA use indicate intracellular conversion of the DHEA into potent androgens, such as testosterone, which are then rapidly converted into inactive metabolites before moving into the circulation. This intracrine conversion of ingested DHEA to active steroids suggests that DHEA intake should provide anabolic effects.
Despite of a lack of change in serum testosterone with DHEA intake, Nestler et al. (95) found that when young men ingested 1,600 mg/day DHEA for 4 weeks, body fat mass, assessed via skinfold measurement, was reduced by 31 percent. However, these results were not duplicated when Welle et al. (92) administered 1,600 mg/day DHEA to men and assessed body composition with more precise measurements (total body water and total body potassium). In addition, ingesting DHEA did not enhance muscle mass or strength gains associated with resistance training (88,96). Therefore, in spite of the potential for DHEA to be converted into testosterone, DHEA intake does not alter body composition or muscle strength in men, and is thus not likely to be an anabolic substance.

*Side Effects with DHEA Intake.* The use of DHEA as a nutritional supplement is associated with hirsutism, acne, reduced serum HDL-C, and insulin resistance in women (89,90,93). In men, it appears that DHEA intake is free from negative side effects (88-92,95,96) aside from elevated estrogens in men over 70 years of age (87).

**Androstenedione**

Androstenedione (Androst-4-ene-3, 17-dione) is formed primarily in the adrenal glands, either from DHEA through 3β HSD (82) or from 17α hydroxyprogesterone via 17,20 lyase (97). Androstenedione has little androgenic or anabolic properties, and appears to be primarily a precursor to other hormones (15,98). Androstenedione is reversibly converted to testosterone via 17βHSD (97), Horton and Tait (23) observed that testosterone converted to androstenedione more readily than androstenedione converted to testosterone.

It is currently unclear whether androstenedione is converted directly into DHT via 5α reductase, or first converted to testosterone and then into DHT. Regardless of the steps in the conversion of androstenedione into DHT, Longcope and Fineberg (16) estimated that 14% of
circulating DHT arises from the $5\alpha$ reduction of androstenedione in adipose tissue alone indicating that androstenedione is a significant precursor for circulating DHT in men.

**Figure 2.** Enzyme kinetics for selected enzymes of androgen formation.

Bruch et al. (102) concluded that the limiting factor in the conversion of androstenedione to DHT is substrate availability and enzymatic kinetics. Androstenedione is also converted into estrone via P450 aromatase (15), and then to estradiol via 17β HSD (97). Longcope et al. (24) observed that the majority of circulating estrogens in men arise from peripheral conversion of androstenedione. The major site for aromatization is adipose tissue;
5α reduction is prostatic tissue, and 17β HSD activity in skeletal muscle. Based on studies in these tissues, the enzyme kinetics for aromatase in adipose tissue \( (K_m = 25 \text{ nmol}\cdot\text{L}^{-1}, V_{\text{max}} = 0.072 \text{ pmol/g/h}) \) (99) and 5α reduction in prostate \( (K_m = 120-211 \text{ nmol}\cdot\text{L}^{-1}, V_{\text{max}} = 0.025 \text{ pmol/g/h}) \) (36,100) of androstenedione favor the production of estrogens and DHT compared to the 17β-HSD conversion of androstenedione to testosterone in skeletal muscle \( (K_m = 1,500 \text{ nmol}\cdot\text{L}^{-1}, V_{\text{max}} = 0.0084 \text{ pmol/g/h}) \) (97,101) (Figure 2). Based on the kinetic data and studies with radiolabeled androstenedione, it appears that androstenedione is not converted appreciably to testosterone in men, and is unlikely to be an effective testosterone-enhancing agent.

While this data on steroidogenic enzyme activity indicates that androstenedione does not alter serum testosterone, but increases DHT and estradiol concentrations, it is important to note that this data does not necessarily reflect the activity of enzymes in vivo. Enzyme activity in vivo can be altered due to transient or localized changes in pH, temperature, the endocrine milieu, or any combination of these. In addition, steroidogenic enzyme activities are altered by age, gender, and vary by tissue (36,97,100). Finally, a favorable \( V_{\text{max}} \) may mitigate an unfavorable \( K_m \). These considerations make it very difficult to predict the response to androstenedione administration without in vivo testing.

**Androstenedione Intake.** In spite of the above-mentioned data, androstenedione as a nutritional supplement has been widely advertised as a testosterone enhancer and anabolic agent. Support for this claim is provided from a single study in 1962, in which serum total testosterone concentrations were increased approximately 5 fold after two women ingested 100 mg androstenedione (3). In addition, a German patent application claimed that a single
dose of 50 mg androstenedione increased serum testosterone by more than 100% and 100 mg increased serum testosterone by more than 300% (4).

In 1998, when Mark McGwire was in the process of setting a Major League Baseball single season home run record, it was discovered that he was using androstenedione. Sales of androstenedione increased dramatically after this revelation (1). In spite of the success of Mr. McGwire and the popularity of androstenedione as a nutritional supplement, there was no scientific evidence evaluating the ergogenic effects of androstenedione intake.

King et al. (5) were the first to report that ingesting 100 mg androstenedione does not acutely increase serum testosterone concentrations in college-aged men or alter serum testosterone concentrations or enhance the gains in muscle mass or strength during an 8 week resistance training program in college aged men. Others have subsequently reported that ingesting androstenedione in doses of 50 – 100 mg does not increase serum testosterone concentrations (6-8,21), enhance the adaptations to resistance training (10,21,96), or promote muscle protein synthesis (8).

In contrast to the reports that 50-100 mg androstenedione intake does not alter serum testosterone concentrations in men (6-8,21), two reports have indicated that androstenedione intake increases serum testosterone when taken in larger doses (6,103). Earnest et al. (103) reported that serum testosterone concentrations were higher during 90 min following the ingestion of 200-mg androstenedione compared with placebo. However, neither free nor total testosterone concentrations were significantly higher at any time point following ingestion of androstenedione. In addition, it appears that the area under the curve, as calculated by these authors, included the area attributable to baseline which was higher during the androstenedione trial. Leder et al. (6) reported that serum testosterone
concentrations in young men were not altered by ingesting 100 mg androstenedione but were increased by 34% during 8 h following a single 300-mg dose. Taken together, the data indicate that serum testosterone concentrations in young men are not altered by androstenedione intake unless the dose is at least 300-mg. There has not been an effort to evaluate any possible age related response to androstenedione intake.

**Side Effects with Androstenedione Intake.** Prolonged androstenedione intake is associated with reduced serum HDL-C (5,10,21) and increased serum estrogen concentrations (5,6,10,21). It appears that androstenedione intake in doses up to 300 mg/day does not change serum LH or FSH concentrations, suggesting no negative hypothalamic-pituitary-adrenal feedback (5,21). Interestingly, androstenedione intake in rats causes enlargement of the aggression center of the brain (104), suggesting that androstenedione intake could cause “Roid Rage” similar to anabolic steroids. However, this effect has not been scientifically investigated in humans. The effect of androstenedione intake on serum DHT or prostate function is currently unknown.

**Androstenediol**

Androstenediol (4-androstene-3β, 17β-diol) is a weak adrenal androgen formed from DHEA via 17β HSD (82,83). The physiologic role of androstenediol appears to be primarily as a precursor to other hormones. Once formed, androstenediol can be converted to testosterone via 3β HSD (83,105) or aromatized to estrogens (105) in the peripheral tissues. The enzyme kinetics for aromatase (K_m=27 nmol•L⁻¹, V_max = 0.09 pmol/g/h) (99) and 3β HSD (K_m=290 nmol•L⁻¹, V_max = 0.0098 pmol/g/h) (101) favor the conversion of
androstenediol to estrogens over testosterone. Currently, no data on the enzyme kinetics for the 5α reduction of androstenediol to DHT are available.

Blaquier et al. (106) reported that 15% of androstenediol is converted to testosterone. However, this research was conducted in tritiated blood and thus did not measure the rate of conversion in peripheral tissue, nor did it measure the conversion of androstenediol to other steroids.

Androstenediol Intake. Based upon the results from Blaquier et al. (106) androstenediol has been advertised as a better alternative for testosterone enhancement, and subsequent gains in muscle mass and strength, than is androstenedione. Although the enzyme kinetics for the conversion of androstenediol to testosterone \((K_m=290 \text{ nmol}\cdot\text{L}^{-1})\) are more favorable than those for the conversion of androstenedione to testosterone \((K_m=1,500 \text{ nmol}\cdot\text{L}^{-1})\) (101), the \(K_m\) is in excess of 10 fold greater than the normal physiologic levels. In addition, the 15% rate of conversion of androstenediol to testosterone reported by Blaquier et al. (106) is nearly the same as the 14% conversion rate for androstenedione to testosterone reported by Horton and Tait (23).

Currently, there have only been 2 studies regarding the use of androstenediol as a nutritional supplement. Earnest et al. (103) reported that ingesting a single dose of 200 mg androstenediol does not acutely increase serum testosterone concentrations in young men. Broeder et al. (10) reported that ingesting 100 mg androstenediol \(b.i.d.\) does not increase serum testosterone concentrations or enhance the adaptations to resistance training in 35-65 year old men.

Side Effects with Androstenediol Intake. Prolonged androstenediol intake causes reduced serum HDL-C and increased serum estrogens (10). There have been no investigations on the
psychological effects of androstenediol intake. The effect of androstenediol intake on serum DHT or prostate function is currently unknown.

**Dihydrotestosterone**

Dihydrotestosterone is a major end product of steroid metabolism, being irreversibly formed from DHEA, androstenedione, androstenediol, and testosterone via 5α reductase (36,40,107). Once formed, DHT binds to the androgen receptor and produces physiologic effects or is excreted in the urine (15).

Dihydrotestosterone binds to the androgen receptor and SHBG more potently than does any other androgen (35,39,108). In addition, DHT is the most potent androgen responsible for the majority of the reproductive tissue development observed during puberty (15,107). Due to the potent androgenicity of DHT, the effect of DHT on the prostate is of tremendous interest. Men with benign prostate hypertrophy (BPH) (41,42), but not prostatic cancer (39), exhibit elevated serum concentrations of DHT.

**Dihydrotestosterone Intake.** Due to the risk of BPH with elevated DHT concentrations, DHT is not readily available as a nutritional supplement. However, since DHT cannot be aromatized, it has been suggested that DHT administration could be a viable treatment for age related sexual dysfunction in men as long as serum estrogen concentrations are not increased (109). Antonio et al. (38) observed that DHT administration in rats produced hypertrophy of muscles involved in reproductive function, but not other skeletal muscles, suggesting that DHT does not produce an anabolic effect in humans.
Estrogens

Estrogens are formed in the ovaries or in peripheral tissue through the aromatization of androgens (31,110). The importance of extragonadal production of estrogens to human health has just recently begun to be understood (29).

There are three primary estrogens in the circulation. Estrone (E1) is formed from the aromatization of androstenedione, and has weak estrogenic effects. Kley et al. (111) observed that there is an increase in aromatization of androstenedione in men who are 20% over ideal body weight. The primary function of E1 is a precursor to estradiol (E2) (15). Estradiol, the most potent circulating estrogen, is formed from the aromatization of testosterone, or from the conversion of estrone through the action of 17β HSD (15,97).

Estriol (E3) is a weak estrogen that can be formed from E2. Serum concentrations of E3 are normally very low in men and women. The primary source of circulating estriol is the placenta (15).

Although estrogens have protective effects for heart disease by enhancing venous compliance and HDL-C synthesis (29,112), elevated serum estrogens in men have been linked to myocardial infarction (113). Elevated serum estrogen concentrations in men have also been linked to gynecomastia (61,114,115). In youth, elevated serum estrogen concentrations can cause premature epiphyseal closure (28). In addition, prostate hypertrophy can occur when serum estrogen and DHT concentrations are elevated (40,43).

Summary

Testosterone is the principal anabolic androgen, while DHEA, androstenedione, and androstenediol primarily function as reversible precursors to other androgens or estrogens.
Dihydrotestosterone is an irreversible 5α reduced end product of androgen metabolism and is the most potent androgen.

Testosterone administration can enhance muscular strength and size, but is banned in most sports, has numerous undesirable, and dangerous, side effects. Androstenedione, androstenediol, and DHEA are marketed as testosterone enhancing/muscle building nutritional supplements. In spite of the advertised claims, intake of testosterone precursors does not enhance muscle strength or serum testosterone concentrations, but does increase serum estrogen and DHT. The enzyme kinetics associated with androstenedione and androstenediol favor the production of end product metabolites, such as DHT and estrogens, rather than testosterone (Figure 2).

Herbal Extracts

As a result of the 1994 Dietary Supplement Health education Act (DHSEA), a large number of herbal products have been marketed as nutritional supplements. Many of these herbal extracts are advertised to enhance athletic performance. Some of these herbal supplements are purported to increase serum testosterone concentrations.

Chrysin and I3C may prevent the aromatization of androgens. Saw palmetto and γ-la may prevent the 5α reduction of androgens. Tribulus terrestris is purported to increase serum LH with a resulting increase in serum testosterone concentrations. By using these herbal extracts in combination with testosterone precursors, the hormonal response to androgen intake may be altered.
Chrysin

Chrysin is a flavonoid from passion flower (Passiflora caerulea). Flavonoids are ubiquitous plant compounds with numerous biological properties. Flavonoids have been found to have estrogenic, antiestrogenic, and aromatase inhibitory capabilities (116).

Flavonoids may prevent the aromatization of testosterone by competing with the steroids for enzyme binding sites. Chrysin has been shown, in vitro, to inhibit P-450 aromatase, the enzyme responsible for aromatization (116). It has been advertised that by inhibiting the aromatization of androgens, testosterone levels will remain high. However, we have recently observed that intake of 675 mg/day chrysin along with 300 mg/day androstenedione does not prevent the increase in serum estrogens associated with androstenedione intake or increase serum testosterone concentrations (21). Since there are age related changes in gonadal function and steroidogenesis, it is possible that older men may respond to chrysin intake differently than do young men.

Indole-3-Carbinol

Indole-3-carbinol is a plant extract from cruciferous vegetables such as cabbage. Indole-3-carbinol inhibits aromatization and enhances urinary estrogen clearance. Ingesting a diet high in I3C (400-500 mg/day) has been observed to reduce serum estrogens in men and women (12-14,117).

It has been advertised that ingesting I3C in conjunction with testosterone precursors will prevent the increased serum estrogens associated with testosterone precursor intake. However, we have recently observed that ingesting 300 mg/day I3C along with 300 mg/day androstenedione does not prevent increased serum estrogens or cause elevated testosterone concentrations in young men (21). Since there are age related changes in gonadal function
and steroidogenesis, it is possible that older men may respond to I3C intake differently than do young men.

**Saw Palmetto**

Saw palmetto (Serenoa repens B) is a type of palm tree found on the southern Atlantic coast of the United States. An extract from the berries of the saw palmetto plant, also called permixon, is used to treat BPH (17).

Saw palmetto extract prevents DHT from binding to the androgen receptor (17,118). Saw palmetto extract has also been observed to prevent estrogen and DHT formation through competitive binding with aromatase and 5α reductase (18,19,119). However, this research has been conducted *in vitro*, which may limit the utility of these findings.

Ingesting 160 mg saw palmetto extract per day does not alter serum testosterone, LH, or FSH concentrations (19). We have recently observed that ingesting 540 mg/day of saw palmetto extract in conjunction with 300 mg/day of androstenedione does not alter the hormonal response to androstenedione intake in young men (21). We were unable to measure serum DHT or prostate specific antigen (PSA) concentrations, and so were unable to evaluate the effect of saw palmetto and androstenedione intake on the 5α reduction of ingested androgens or prostate health.

**Tribulus terrestris (Genus species)**

Tribulus terrestris is a plant commonly known as puncture vine. Ingesting TT extract is advertised to enhance serum testosterone concentrations by increasing serum LH concentrations. Although the effects of ingesting TT alone on serum testosterone and LH concentrations has not been investigated, we have recently observed that ingesting 750 mg/day of TT along with 300 mg/day androstenedione does not alter serum testosterone or
LH concentrations (21). However, the effect of ingesting TT and testosterone precursors has not been investigated in older men.

**Gamma Linolenic Acid**

Gamma linolenic acid is a C-18 unbranched fatty acid with three double bonds at positions 6, 9, and 12, all in the cis configuration. Liang and Liao (20) reported that, in rat liver microsomes, concentrations of ~10 μM γLA both decreased the $V_{\text{max}}$ and increased the $K_m$ of 5α reductase without altering the activity of 17β HSD. It is difficult to classify this type of inhibition, since competitive inhibitors increase the $K_m$ while non-competitive inhibitors decrease the $V_{\text{max}}$, suggesting that γLA is a universal inhibitor of 5α reductase. In addition, this research was conducted *in vitro*, which limits the applicability of this research to nutritional supplements *in vivo*.

**Summary**

Numerous herbal extract are marketed to promote testosterone formation. Although chrysin, I3C, saw palmetto, γLA, and TT are advertised to alter steroid formation in favor of increased serum testosterone concentrations, most of these products have not been investigated *in vivo*. In addition, while these products have been shown effective *in vitro*, and not in conjunction with exogenous androgen administration. Therefore, the utility of chrysin, I3C, saw palmetto, γLA, and TT to prevent changes in serum estrogens and DHT associated with testosterone precursor intake needs to be evaluated.

We have recently observed that when chrysin, I3C, saw palmetto, and TT are taken in conjunction with androstenedione and DHEA in young men, there is no change in the hormonal response compared to that when taking only androstenedione. However, since there is an age related change in serum steroid concentrations, ingesting chrysin, I3C, saw
palmetto, γLA, and TT may alter the hormonal response to testosterone precursor intake in older men.

**Conclusion**

In spite of the popularity of testosterone precursor nutritional supplements, these products do not cause physiologically significant changes in serum testosterone concentrations. Much of the research involving the use of testosterone precursors has used young men who already have high endogenous testosterone concentrations. Since serum testosterone concentrations decline with age, and basal endogenous testosterone concentrations may alter the hormonal response to testosterone precursor intake, one purpose of this project is to determine if age influences the hormonal response to testosterone precursor intake.

There are currently numerous herbal extracts that are claimed to alter enzyme function and prevent the aromatization or 5α reduction of androgens. Most of these products have not been tested *in vivo* or in the presence of exogenous androgens. Another purpose of this project is to determine if combining herbal extracts with testosterone precursors alters the hormonal response to nutritional supplements.

Ingested androgens are subjected to a large amount of digestive and hepatic catabolism, limiting the amount of testosterone precursor that enters the extra hepatic circulation, which may prevent the formation of testosterone from the ingested androgen. A final purpose of this project is to determine if sublingual cyclodextrin androstenediol increases serum testosterone concentrations in men.
References


89. Morales, A. J., R. H. Haubrich, J. Y. Hwang, H. Asakura, and S. S. Yen. The effect of six months treatment with a 100 mg daily dose of dehydroepiandrosterone (DHEA)


98. Saartok, T., E. Dahlberg, and J. A. Gustafsson. Relative binding affinity of anabolic-androgenic steroids: comparison of the binding to the androgen receptors in skeletal muscle and in prostate, as well as to sex hormone-binding globulin. *Endocrinology.* 114: 2100-2106, 1984.


CHAPTER 3. ENDOCRINE RESPONSES TO CHRONIC ANDROSTENEDIONE INAKE IN 30-56 YEAR OLD MEN


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5Contributed significantly to sample collection and overall research completion

Abstract

In young men chronic ingestion of 100 mg androstenedione three times per day does not increase serum total testosterone, but does increase serum estrogen and androstenedione concentrations. We investigated the effects of androstenedione ingestion in healthy 30–56 year old men. In a double blind, randomly assigned manner, subjects consumed 100 mg androstenedione three times daily (ASD; n=28) or placebo (PL; n=27) for 28 days. Serum androstenedione, dihydrotestosterone, free and total testosterone, estradiol, prostate specific antigen, and lipid concentrations were measured at week 0, and each week throughout the supplementation period. Serum total testosterone and prostate specific antigen
concentrations did not change with supplementation. Elevated serum concentrations of androstenedione (300%), free testosterone (45%), dihydrotestosterone (83%), and estradiol (68%) were observed during weeks 1-4 in ASD (P<0.05). There was no relationship between age and changes in serum androstenedione ($r^2=0.024$), free testosterone ($r^2=0.00$) or estradiol ($r^2=0.029$) concentrations with ASD, while the serum dihydrotestosterone response to ASD ingestion was related to age ($r^2=0.244$; P<0.05). Serum concentrations of HDL-C were decreased by 10% during the supplementation period (P<0.05). These results suggest that the ingestion of 100 mg androstenedione three times per day does not increase serum total testosterone or PSA concentrations, but does elicit increases in androstenedione, free testosterone, estradiol, dihydrotestosterone, and decreases serum HDL-C concentrations.

Introduction

Recently, the ingestion of pro-hormone nutritional supplements has been purported to increase serum testosterone concentrations. In two women, ingestion of a single 100-mg dose of androst-4-ene-3, 17-dione (androstenedione) elevated serum testosterone concentrations approximately 600% within 60 min (1). However, in young men ingestion of 100- or 200 mg of androstenedione does not change serum testosterone concentrations (2-6) while ingesting a single 300-mg dose of androstenedione promotes a smaller (34%) increase in serum total testosterone concentrations that persists for 4-6 h (5). Women have lower basal serum testosterone concentrations than men and serum testosterone concentrations decline with age in men (7-9). Therefore, it is possible that basal serum testosterone concentrations, as well as age, may influence the effects of androstenedione ingestion on serum testosterone concentrations.
Recently, Wallace et al. (10) examined the effects of ingesting 100 mg/day androstenedione for 12 wk on serum testosterone, DHEA, androstenedione, prostate specific antigen (PSA) and insulin-like growth factor-1 in 40–60 year old men. The hormonal and lipid response to a larger dose of androstenedione in older men is unknown. Therefore, we evaluated the effects of 100 mg androstenedione three times per day four 28 days on serum free testosterone, total testosterone, estradiol, dihydrotestosterone, prostate specific antigen, and serum lipid concentrations in 30 – 56 year old men. Wallace et al. (10) found no differences in measurements of well-being and libido between placebo and 100- mg·day\(^{-1}\) androstenedione ingestion in older men. Therefore, we also evaluated the effect of chronic androstenedione ingestion on perception of mood states in 30-56 year old men.

**Methods**

**Subjects**

Fifty-six men, aged 30 through 56, were recruited from the university and local community to participate in this project. All subjects signed an informed consent and completed a written medical history to eliminate any subjects with any known chronic disease. Prior to participating in this study subjects were questioned to ensure they were not currently or previously using nutritional supplements. The Iowa State University human subjects review board approved this study. Subjects were stratified into age groups representing the fourth (30 year olds, n=20), fifth (40 year olds, n=20), and sixth (50 year olds, n=16) decades.

**Supplementation**

Subjects were randomly assigned in a double blind, counter balanced fashion to treatment groups consuming capsules containing either placebo (PL) or 300 mg·day\(^{-1}\).
androstenedione in doses of 100 mg taken three times daily (ASD; Experimental and Applied Sciences, Golden, CO). An independent laboratory (Integrated Biomolecule, Tuscon, AZ) verified purity (~99%) and content of the androstenedione capsules via high performance liquid chromatography (HPL-C). Subjects were instructed to consume the supplement capsules daily before 9:00 am, at 3:00 pm, and before bedtime in equal doses throughout the 4 weeks of supplementation. Compliance was monitored through written records of supplementation and the return of unused supplements at the completion of the study.

**Diet and Activity**

Subjects were instructed to maintain their normal diet and activity patterns throughout the 4-week study period. Subjects were given verbal and written instructions regarding the reporting of dietary intake and instructed to maintain a diet, medication, and exercise record for the 2 days prior to each blood sampling. The diet and exercise records were collected prior to weekly blood sampling and analyzed using commercial software (Nutritionist 4, N-Squared Computing, San Bruno CA).

**Blood Sample Collection and Analysis**

Fasting blood samples were collected between 6:30 and 8:00 a.m. once per week on the same day each week. Subjects reclined while blood samples were obtained without stasis from an antecubital vein. Blood samples were immediately placed in an ice bath until centrifugation and serum separation. Blood samples were analyzed for lipid, glucose, enzyme, and chemical composition by a commercial laboratory (Quest Diagnostics, Wood Dale, IL). Serum concentrations of estradiol, total testosterone, free testosterone, and androstenedione were measured with commercial radioimmunoassay kits (Diagnostic Products Corp, Los Angeles, CA, and Diagnostic Systems Laboratory, Webster, TX).
Commercially available enzyme-linked immunosorbent assays (ELISA) were used to measure serum concentrations of prostate specific antigen (PSA; Bio-Clin, Inc, St. Louis, MO) and dihydrotestosterone (DHT; Immuno-Biological Laboratories, Hamburg, Germany). All samples for each subject were analyzed in duplicate within the same assay and the intra-assay coefficients of variation for total testosterone, free testosterone, estradiol, androstenedione, DHT and PSA were 6.1%, 7.2%, 6.6%, 5.8%, 3.2% and 6.6%, respectively. According to the suppliers of the RIA and ELISA kits, there is no detectable cross reactivity of the assays for androstenedione, DHT, estradiol or testosterone.

Profile of Mood States

Subjects completed and returned questionnaires each week assessing perceptions of health and well-being (11). Questionnaires consisted of yes or no questions assessing changes in libido, symptoms of illness, or mood change for the 3 days prior to blood sampling.

Calculations and Statistics

Data were analyzed using commercial software (SPSS Inc, Chicago, IL). Statistical analyses of age group effects were performed using a 3 factor (week by supplement by age group) repeated measures analysis of variance (ANOVA). Specific mean differences (P < 0.05) were identified using Student Newman-Keuls post hoc comparisons. Relationships between the effects of supplementation and measured variables were analyzed using simple linear regression. The percent change in serum hormones and blood lipids was calculated as the mean percent change in serum concentrations during weeks 1-4. Data are presented as means ± SE.
Results

Subjects

Subjects came from diverse occupations and physical activity levels. Regular participation in aerobic exercise such as walking, jogging and racquetball was reported in 10 subjects in PL and 12 subjects in ASD while 6 subjects in PL and 5 in ASD reported regular participation in resistance training. Subjects reported no changes in their day-to-day physical activity pattern throughout the study duration including the day prior to blood sampling. Height, body mass, and body mass index (BMI) were not different between treatment or age groups (Table 1). Body mass and BMI were not altered by supplementation. One 50-year-old subject in the placebo group was diagnosed with non-insulin dependent diabetes mellitus during the study, informed of his condition, and his data have been excluded from all analyses.

Dietary Analysis

There were no significant age or treatment group differences in dietary energy, protein, carbohydrate, total fat, saturated fat, or polyunsaturated fat intake.

Hormonal Response to Supplementation

Basal serum androstenedione levels were higher in the 30 year olds (P < 0.05) than in the 40 or 50 year olds (Figure 1). Ingestion of androstenedione resulted in significant and similar mean increases of 268%, 300% and 357% in serum androstenedione concentrations throughout the 4 weeks of supplementation for 30, 40, and 50 year olds, respectively (P < 0.05). Mean changes in serum androstenedione concentrations in ASD during weeks 1-4 were not correlated to age ($r^2$=0.024) or BMI ($r^2$=0.00).
Serum total testosterone concentrations were not different among age or treatment groups before or during the 4 weeks of supplementation (Figure 2). There was also no age related effect of supplementation on serum total testosterone concentrations ($r^2=0.018$).

Basal serum free testosterone concentrations were higher in the 30 year olds than in the 50 year olds ($P<0.05$; Figure 3). Ingestion of androstenedione resulted in elevated serum concentrations of free testosterone during weeks 1-4 ($P<0.05$) by 37%, 51%, and 46% for the 30, 40 and 50 year olds, respectively. The change in serum free testosterone concentrations was related to basal free testosterone concentrations ($r^2=0.284$) but not to age ($r^2=0.00$) or BMI ($r^2=0.0379$).

Baseline serum DHT levels were higher in the 30 year olds than in the 40 or 50 year olds (Figure 4). Serum DHT was unchanged throughout the 4-week supplementation period in PL, while ingestion of androstenedione increased ($P<0.05$) serum DHT concentrations in the 30, 40, and 50 year olds by 56%, 81% and 113%, respectively, throughout the 4-week supplementation period. There was a significant ($P<0.05$) relationship between age and the mean increase in serum DHT during the four weeks of androstenedione supplementation ($r^2=0.244$). Body mass index did not correlate with changes in serum DHT ($r^2=0.023$).

Basal serum estradiol concentrations were not effected by age. Serum estradiol concentrations were significantly and similarly increased throughout weeks 1-4 by 80%, 55%, and 71% in ASD for 30, 40, and 50 year olds, respectively (Figure 5). There was no relationship between age and the mean increase in serum estradiol throughout weeks 1-4 in ASD ($r^2=0.029$). The mean increase in serum estradiol concentrations throughout weeks 1-4 was also unrelated to the BMI ($r^2=0.133$).
Serum PSA concentrations (Figure 6) were initially higher (P<0.05) for the 50 year olds taking ASD (4.4 ± 0.6 ng·ml⁻¹) than any other age or supplement group (2.5 ± 0.4 ng·ml⁻¹ for all others combined). However, serum PSA concentrations were not altered during the four weeks of supplementation.

Serum Lipid Response

Serum HDL-C levels were reduced by 10% (P<0.05) at week 1 in ASD and remained depressed throughout the remainder of the 4 weeks of supplementation, with no observed age related effect (r²=0.037; Table 2). There was a significant inverse correlation between the mean changes throughout week 1-4 in serum androstenedione and HDL-C concentrations (r²=0.284). Concentrations of serum low-density lipoprotein cholesterol (LDL-C), total cholesterol (Total-C), and the total cholesterol/HDL cholesterol ratio were not changed in either ASD or PL.

Blood Chemistry Response

Serum concentrations of gamma-glutamyltransferase, aspartate aminotransferase and alanine aminotransferase were unchanged throughout the 4-week supplementation period and were not affected by age. There were also no age or supplement related change in concentrations of serum protein, albumin, globulin or other indices of blood chemistry.

Profile of Mood States

Decreased libido was reported on one occasion by two subjects in PL and one occasion by one subject in ASD during the 4-week supplementation period while increased libido was reported 6 times by 4 subjects in PL and 8 times by 4 subjects in ASD suggesting that libido is not altered by androstenedione ingestion. There were no differences between PL and ASD in the frequency of reported changes in energy level, memory, stress, appetite,
chest pain, headaches, or overall sense of health. The most commonly reported side effect of supplementation was heartburn, with an increased frequency of heartburn reported on 13 occasions by 10 subjects in ASD and five occasions by five subjects in PL.

**Discussion**

The age at which serum testosterone concentrations in men are significantly reduced is unclear. Some reports indicate significant decreases in serum total testosterone beginning in the fifth (9) or sixth (8) decades of life, while others report relatively stable serum total testosterone concentrations through the seventh decade of life (7). There is also disagreement regarding the age at which serum free testosterone begins to decline in men (7-9). However, the current results are in agreement with previous reports that observed very small declines in serum total testosterone concentrations with age, while serum free testosterone concentrations decline much earlier in life. The age related decline in serum free testosterone concentrations while serum total testosterone concentrations remain stable is associated with an age-associated increase in sex hormone binding globulin (SHBG) concentrations (7-9).

Two recent studies concluded that serum total testosterone concentrations in men increase after ingestion of androstenedione (5;12). Earnest et al. (12) reported that the incremental area under the curve for serum testosterone concentrations during 90 min following the ingestion of 200-mg androstenedione was higher compared with placebo. However, neither free nor total testosterone concentrations were significantly higher at any time point following ingestion of androstenedione. In addition, the values reported for the area under the curve apparently included the area attributable to baseline serum testosterone concentrations, which were slightly higher in the group of subjects ingesting
androstenedione. In agreement with the present results as well as our previous findings (3;4) Leder et al. (5) observed that ingestion of 100-mg androstenedione did not increase serum total testosterone concentrations. In contrast, these authors observed that serum testosterone concentrations during 8 h following a single 300-mg androstenedione dose increased by 34%. Since blood samples in the current study were collected ~10 h after the previous 100 mg dose of androstenedione it was not possible to assess peak hormonal changes after androstenedione ingestion. Our previous research indicates that after ingesting 100 mg androstenedione, peak increases in serum androstenedione concentrations occur ~120-300 min after ingestion, while changes in serum estradiol concentrations are not evident during the 6 h after ingestion, and serum total testosterone concentrations are not altered (3;4). Although transient increases in serum total testosterone after ingestion of 100 mg of androstenedione cannot be ruled out, the present results support our previous findings (3;4) and those of others (2;5;10) that total testosterone concentrations are not chronically increased by ingestion of androstenedione in doses of up to 300 mg per day, when taken in 100 mg doses. The current study extends these findings by demonstrating that ingestion of 100 mg of androstenedione three times daily does not alter serum total testosterone concentrations in middle-aged men.

A novel finding was that while serum total testosterone was unaffected, serum free testosterone increased by 37-51% in these 30-56 year old men. These results are in contrast to our previous finding that 100 mg of androstenedione three times daily does not chronically increase serum free testosterone concentrations in 23 year old men (3;4). Consistent with the previously observed age related declines in serum free testosterone concentrations (7-9), the serum free testosterone concentrations in the current subjects were lower than in the younger
subjects in our previous research (3;4). In addition the mean increase in serum free testosterone concentrations during weeks 1-4 was significantly related to basal serum free testosterone concentrations in the current study. These findings suggest that oral androstenedione ingestion may promote increases in serum free testosterone concentrations in men with low serum free testosterone concentrations.

The finding of unchanged serum total testosterone, albumin, and protein concentrations, together with the significant increase in free testosterone concentrations suggests that ingestion of 100 mg androstenedione three times daily changes the concentration of SHBG bound testosterone. Although androstenedione is a weaker androgen than testosterone (13), exogenous testosterone administration decreases serum SHBG concentrations (14). In addition, since SHBG may have a greater binding affinity for DHT than for testosterone (15), it is also possible that the increase in serum free testosterone concentrations we observed was a result of a decoupling of SHBG from testosterone to bind with DHT or other steroids.

The metabolic significance of the transient increase in serum total testosterone concentrations found by Leder et al. (5) and the chronic elevation of free testosterone in the present study is uncertain. Increased rates of muscle protein synthesis (16) and increased muscular strength (14; 16) have been observed after testosterone administration resulting in very large increases in serum testosterone concentrations (~100-600%). It is unknown whether more prolonged, small elevations in serum testosterone concentrations of the magnitude observed in the current study and by Leder et al. (5) would produce measurable effects on muscle size and strength.
The significant increase in the serum DHT concentrations observed in the current study suggests that a significant amount of the elevated serum androstenedione and free testosterone underwent conversion to DHT. The conversion of androstenedione to dihydrotestosterone can occur in prostate, skin, or adipose tissue, which all contain appreciable concentrations of 5α-reductase (13; 17; 18). Longcope and Fineberg (17) estimated that 14% of total serum androstenedione is converted to dihydrotestosterone in adipose tissue alone. Serum androstenedione concentrations increased by ~19 nmol·L⁻¹, and serum DHT concentrations increased by ~1,500 pmol·L⁻¹ in the present study, corresponding to an 8% conversion of androstenedione to DHT. Although the increases in serum DHT concentrations were weakly related to age (R²=0.24) consistent with age related increases in 5α-reductase concentrations (19), the mean DHT concentrations were not different during weeks 1-4 in the three age groups. These findings suggest that androstenedione ingestion will increase serum DHT concentrations in men of all ages.

Although DHT is the most potent naturally occurring androgen, the metabolic effects of DHT appear to occur primarily in reproductive organs, since skeletal muscle tissue does not contain appreciable quantities 5α-reductase (13; 17). Recently, it has been observed that the administration of DHT to castrated rats restored the levator ani and bulbocavernosus muscles to pre-castration size but had no effect on the size of plantaris muscle (20), supporting the notion that the anabolic effect of DHT is limited to tissues involved with reproduction. Although we did not measure serum DHT in our previous study in which androstenedione intake did not alter muscle size or strength in 19-29 year old men (4), it is likely that serum DHT concentrations during supplementation were similar to those observed in the present study. Taken together, these findings suggest that the increases in circulating
DHT observed in the current study are unlikely to have any anabolic effects on skeletal muscle of older men.

In agreement with previous observations in young men (2-6), ingestion of androstenedione caused a significant increase in serum estradiol concentrations in the middle-aged-men in the current study providing evidence that a portion of ingested androstenedione is aromatized (21;22). Aromatization can occur in a number of tissues, including adipose tissue (21;23;24). While adipose tissue contains aromatase (23) and the degree of obesity has been related to enhanced aromatization of androstenedione (25), we found no relationship between the change in serum estradiol concentrations and body mass or BMI. While our subjects were slightly overweight (110% of ideal body weight), the aromatization of androstenedione may not be affected by adiposity until 120% of ideal body weight is reached (30). In addition, the enzyme kinetics for the aromatization of androstenedione \( (K_m=25 \text{ nmol·L}^{-1}) \) (23) favor the production of estrogens compared to the 17β-HSD conversion of androstenedione to testosterone \( (K_m=1,500 \text{ nmol·L}^{-1}) \) (26).

The reduction in serum HDL-C associated with ingestion of 100 mg androstenedione three times per day is in agreement with our previous research in young men (3;4). A decrease of 0.13 mmol·L\(^{-1}\) in serum HDL-C concentrations corresponds to a 10-15% increase in the risk of atherosclerotic lesion development (27) and heart disease (28). While ingestion of 50 mg androstenedione twice daily for 12 weeks does not alter serum HDL-C in middle aged men (10), ingestion of 100 mg androstenedione three times daily reduces serum HDL-C concentrations by 15% (present study;3;4), suggesting that the dosage of androstenedione may affect the serum lipid response to androstenedione ingestion.
While the initial serum PSA concentrations in the 50 year olds consuming ASD were above average and may reflect altered prostate function, serum PSA concentrations were not changed by supplementation in any age group. The use of PSA measurements to evaluate prostate function, however, should be viewed cautiously, For example, it has been reported that 30% of patients with prostatic tumors present with normal PSA concentrations, while elevated PSA concentrations are found in ~2% of healthy men (29). In addition, since elevated serum DHT and estradiol concentrations may cause benign prostate hypertrophy (30), and the blockade of adrenal androgens is advised for the remediation of benign prostate hypertrophy (18) it is possible that more prolonged androstenedione supplementation may result in detectable changes in prostate function.

Ingesting 50 mg androstenedione twice daily for 12 weeks did not change perceived health or mood in middle aged men (10). In the present study there was no difference in the perceptions of mood, health, or libido between subjects ingesting placebo or androstenedione. Taken together, these results suggest that short-term ingestion of androstenedione at the doses of 50 mg twice daily, or 100 mg three times daily, are associated with no psychological or emotional benefits.

The effects of the 55-80% elevation in estradiol concentrations in the current study are not clear. Although estrogens may produce favorable changes in lipid profiles and cardiovascular reactivity (31), a 21% higher serum estradiol concentration has been observed in male subjects experiencing myocardial infarction compared to those with no heart disease (32). Increased serum estradiol levels in men have been associated with the development of gynecomastia (24). While a cause and effect relationship has not been established, increased serum androstenedione concentrations have also been observed in patients with pancreatic
carcinoma (33). Thus, the changes in the hormonal milieu associated with androstenedione ingestion may increase the risk for additional adverse health consequences.

While there has been speculation that consumers of androstenedione use doses much higher than have been studied (34), the dosage of androstenedione used by consumers and the effects of larger doses of androstenedione are unknown. Moreover, it is likely that any possible benefit of raising serum testosterone concentrations by ingesting higher doses of androstenedione would be associated with larger increases in serum estradiol, DHT, and a larger decrease in serum HDL-C concentrations.

In summary, ingestion of 100 mg androstenedione three times per day did not alter serum total testosterone concentrations in 30-56 year old men. Ingestion of androstenedione produced elevated serum free testosterone, androstenedione, estradiol, and dihydrotestosterone concentrations, and reduced serum HDL-C concentrations. There was also no change in perceived mood, health, or libido associated with androstenedione ingestion in 30-56 year old men. Our results suggest that ingesting 100 mg androstenedione three times daily is unlikely to provide a desirable hormonal milieu for promoting increases in muscle size, and may lead to untoward health effects.

References


Tables and Figures

Table 1 Anthropometric data for subjects

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Values are expressed as means ± SE.
Table 2  Serum lipid concentrations during 4 weeks of supplementation

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Values are expressed as means ± SE (mmol/L). * Significantly different from week 0 (Main treatment effect; P<0.05)
Figure 1. Serum androstenedione concentrations during four weeks of nutritional supplementation.

* = significantly different from week 0 (Main group effect, P < 0.05). † = 40 and 50 year olds different from 30 year olds (Main group effect, P < 0.05).
Figure 2. Serum total testosterone concentrations during four weeks of nutritional supplementation.
Figure 3  
Serum free testosterone concentrations during four weeks of nutritional supplementation.

* = significantly different from week 0 (Main group effect, P < 0.05). † = 50 year olds different from 30 year olds (Main effect, P < 0.05).
* = significantly different from week 0 (Main group effect, P < 0.05). † = 40 and 50 year olds different from 30 year olds (Main effect, P < 0.05).
Figure 5. Serum estradiol concentrations during four weeks of nutritional supplementation.

* = significantly different from week 0 (Main effect, P < 0.05)
Figure 6. Serum PSA concentrations during four weeks of nutritional supplementation.

* = significantly different from all other age and treatment groups) main group effect, P<0.05.)
CHAPTER 4. EFFECTS OF ANDROSTENEDIONE-HERBAL SUPPLEMENTATION ON SERUM SEX HORMONE CONCENTRATIONS IN 30-59 YEAR OLD MEN


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⁵Contributed significantly to sample collection and overall research completion

Abstract

The effectiveness of a nutritional supplement designed to enhance serum testosterone concentrations and prevent the formation of dihydrotestosterone and estrogens from the ingested androgens was investigated in healthy 30 to 59 year old men. Subjects were randomly assigned to consume DION (300-mg androstenedione, 150-mg dehydroepiandrosterone, 540-mg saw palmetto, 300-mg indole-3-carbinol, 625-mg chrysin, and 750-mg Tribulus terrestris per day; n=28) or placebo (n=27) for 28 days. Serum free
testosterone, total testosterone, androstenedione, dihydrotestosterone, estradiol, prostate specific antigen, and lipid concentrations were measured before and throughout the 4-week supplementation period. Serum concentrations of total testosterone and prostate specific antigen were unchanged by supplementation. DION increased (P<0.05) serum androstenedione (342%), free testosterone (38%), dihydrotestosterone (71%), and estradiol (103%) concentrations. Serum HDL-C concentrations were reduced by 5.0 mg/dl in DION (P<0.05). Increases in serum free testosterone (r² = 0.01), androstenedione (r² = 0.01), dihydrotestosterone (r² = 0.03), or estradiol (r² = 0.07) concentrations in DION were not related to age. While the ingestion of androstenedione combined with herbal products increases serum free testosterone concentrations in older men, these herbal products do not prevent the conversion of ingested androstenedione to estradiol and dihydrotestosterone.

Introduction

Androstenedione ingestion in dosages of 100-300 mg·day⁻¹ in young men does not chronically increase serum free or total testosterone concentrations, but does increase serum estrogen concentrations [1-4]. In contrast, we recently observed 100 mg androstenedione taken three times daily in older (30-56 y) men for 28 days produces a modest (45%) increase in serum free testosterone and more substantial elevations in serum dihydrotestosterone (83%), and estradiol (68%) [5]. Although these hormonal increases were significant, the resultant concentrations remained within the normal physiological range.

Recently, it has been suggested that certain herbal extracts may prevent the conversion of androgens to dihydrotestosterone (DHT) and estrogens, and increase serum testosterone concentrations. Although it has been advertised that an extract from Tribulus terrestris increases serum luteinizing hormone and testosterone concentrations, there are no
published studies confirming this claim. Indole-3-carbinol, an extract from cruciferous vegetables, has been shown to enhance oxidative metabolism and excretion of estrogens in middle-aged men and women [6]. Chrysin, a flavonoid from Passiflora caerulea, inhibits the aromatization of androgens to estrogens in vitro [7]. Saw palmetto decreases the in vitro 5α reduction of androgens [8]. It has been speculated that the ingestion of these extracts with DHEA and androstenedione may prevent 5α-reduction and aromatization, limiting the fate of the ingested androgens to the 17β hydroxysteroid dehydrogenase (17β-HSD) pathway [9]. However, we have observed that when young men ingest a supplement (DION) containing androstenedione, dehydroepiandrosterone (DHEA), indole-3-carbinol, chrysin, saw palmetto, and Tribulus terrestris the hormonal response is not different from ingesting androstenedione alone [10]. Since we have previously observed that the testosterone response to androstenedione ingestion is different in older and younger men [1,5], we tested the hypothesis that inclusion of these herbal extracts during chronic androstenedione and DHEA supplementation alters the fate of the ingested androgens in older men.

**Methods**

**Subjects**

Fifty-six healthy men between the ages of 30 and 59 were recruited from the university and local community for this project. All subjects signed an informed consent and completed a thorough written medical history to remove any subjects with known chronic disease. Subjects were questioned to ensure they had no current or previous use of nutritional supplements prior to participating in this study, which was approved by the Iowa State University human subjects review board.
Blood Sample Collection and Analysis

Fasting blood samples were collected between 6:30 and 8:00 a.m. at the same time each week. Five minutes after insertion of a catheter into an antecubital vein, blood samples were obtained without stasis from supine subjects. Blood samples were immediately placed in an ice bath until centrifugation and serum separation. A commercial laboratory performed blood chemistry analysis of liver function enzymes, serum albumin, proteins, globulins, and lipid concentrations (Quest Diagnostics, Wood Dale, IL). Serum concentrations of estradiol, free testosterone, total testosterone, and androstenedione were measured in duplicate via commercial radioimmunoassay kits (Diagnostic Products Corp, Los Angeles, CA and Diagnostic Systems Laboratory, Webster TX). Commercially available enzyme-linked immunosorbent assays were used to measure serum concentrations of prostate specific antigen (PSA; Bio-Clin, Inc, St. Louis, MO) and dihydrotestosterone (DHT; Immuno Biological Laboratories, Hamburg, Germany). The intra assay coefficients of variation for total testosterone, free testosterone, estradiol, androstenedione, DHT and PSA were 6.1%, 7.2%, 6.6%, 5.8%, 3.2% and 6.6%, respectively. According to the information supplied by the assay manufacturers, there was no detectable cross reactivity between androstenedione and free testosterone, total testosterone, DHT, or estradiol.

Diet and Activity

Subjects were instructed to maintain normal diet and activity patterns throughout the study. Subjects were given verbal and written instructions on the reporting of daily physical activity and dietary intake and maintained diet, medication, and exercise records for the 2 days prior to each blood sampling. The dietary records were collected prior to weekly blood
sampling and analyzed using commercial software (Nutritionist 4, N-Squared computing, San Bruno CA).

**Supplementation**

Using a double blind counter balanced design subjects were randomly assigned to consume capsules containing rice flour placebo (PL) or DION that contained daily doses of 300 mg androstenedione, 150 mg DHEA, 540 mg saw palmetto, 300 mg indole-3-carbinol, 625 mg chrysin, and 750 mg Tribulus terrestris. Subjects were instructed to consume the supplements in equal doses before 9:00 am, at 3:00 pm, and before bedtime. A supplier of nutritional supplements (Experimental and Applied Sciences, Golden, CO) provided the DION and an independent laboratory (Integrated Biomolecule, Tucson, AZ) verified purity and content of the supplements with high performance liquid chromatography (HPLC). Compliance was monitored through written records of supplementation and the return of empty supplement containers at the completion of the study.

**Calculations and Statistics**

To facilitate data presentation, subjects were stratified into age groups representing the fourth (30 year olds), fifth (40 year olds), and sixth (50 year olds) decades. Data were analyzed using commercial software (SPSS Inc, Chicago, IL). Statistical analyses were performed using a 3 factor (Week by Treatment by Age group) repeated measures analysis of variance (ANOVA). Because basal serum hormone concentrations may alter the endocrine response to androgen supplementation, analysis of covariance (ANCOVA) was performed using basal hormone concentrations as the covariate. Specific mean differences ($P < 0.05$) were analyzed using a Student Newman-Keuls multiple comparison test. Analysis of age
related effects of supplementation were conducted through tests of simple linear regression. Data are presented throughout as means ± SE.

Results

Subjects

Subjects came from a variety of occupations and had varied physical activity levels. Nine subjects in DION and 10 in PL reported regular participation in aerobic activity while resistance exercise was reported in 5 subjects in DION and 6 in PL. Physical activity was reported in 55% of the 30 and 40 year olds and in 20% of the 50 year olds. No changes in physical activity patterns were reported during the course of the investigation. No differences in height, body mass, or body mass index (BMI) were found between treatment and age groups (Table 1), and body mass and BMI did not change in any age or treatment group during the experimental period. One 50-year-old subject in the placebo group was diagnosed with non-insulin dependent diabetes mellitus during the study and informed of the condition; his data have been excluded from all analyses. This project was part of a larger study and the data for the placebo group have been reported elsewhere [5].

Hormonal Response to Supplementation

The results of the ANCOVA revealed no significant effect of basal serum hormone concentrations on the endocrine response to DION ingestion. Since the results of the statistical analysis were not different between the ANOVA and ANCOVA, the actual means rather than the adjusted means from the ANCOVA, are presented throughout the text.

Serum total testosterone concentrations did not differ significantly between the three age groups, and were not altered during the 4 weeks of supplementation in any age or treatment group (Figure 1).
Basal serum free testosterone concentrations were significantly related to age ($r^2=0.23$), and were lower in the 50 year olds than the 30 year olds (Figure 2; $P<0.05$). There was a significant ($P < 0.05$) Treatment-by-Week effect, but no Age-by-Week-by-Treatment effect. For all ages combined, ingestion of DION resulted in a 38% increase ($P<0.05$) in serum free testosterone concentrations during weeks 1-4. There was no relationship between the observed change in serum free testosterone concentrations and age ($r^2=0.01$).

Basal serum androstenedione concentrations were higher in the 30 year olds than in the 40 or 50 year olds (Figure 3; $P < 0.05$). There was a significant ($P < 0.05$) Treatment-by-Week effect, but no Age-by-Week-by-Treatment effect. For all ages are combined, ingestion of DION resulted in a 342% increase in serum androstenedione concentrations during weeks 1-4. There was no relationship between the observed change in serum androstenedione concentrations and age ($r^2=0.01$). Serum androstenedione concentrations were increased above normal expected values in response to DION ingestion.

Baseline serum DHT concentrations were higher in the 30 year olds than in the 40 or 50 year olds ($P<0.05$; Figure 4). There was a significant ($P < 0.05$) Treatment-by-Week effect, but no Age-by-Week-by-Treatment effect. For all ages combined, ingestion of DION resulted in a 71% increase in serum DHT concentrations during weeks 1-4. There was no relationship between the observed change in serum DHT concentrations and age ($r^2=0.047$).

Basal serum estradiol concentrations were not different between age or treatment group (Figure 5). There was a significant ($P < 0.05$) Treatment-by-Week effect, but no Age-by-Week-by-Treatment effect. For all ages combined, ingestion of DION resulted in a 103% increase in serum estradiol concentrations during weeks 1-4. No relationship was observed between the mean increase in serum estradiol concentrations during weeks 1-4 and age.
(r²=0.012) or body mass index (r²=0.071). The change in serum estradiol concentrations was not different between physically active and inactive subjects.

For all ages combined the ratio of total testosterone (nmol·l⁻¹) to estradiol (nmol·l⁻¹) decreased (P<0.05) during weeks 1-4 in DION (134 ± 15 vs. 65 ± 5) and remained stable in PL (106 ± 9 vs. 113 ± 9). Similarly, for all ages combined, the ratio of free testosterone (pmol·l⁻¹) to estradiol (pmol·l⁻¹) decreased (P<0.05) during weeks 1-4 in DION (0.42 ± 0.04 vs. 0.29 ± 0.02) and did not change in PL (0.37 ± 0.03 vs. 0.39 ± 0.03).

Serum PSA concentrations were unaffected by age or supplementation (Figure 6). The mean serum PSA concentrations throughout the 4 weeks of supplementation for all treatment groups combined were 2.1 ng·ml⁻¹, 2.6 ng·ml⁻¹ and 2.5 ng·ml⁻¹ for the 30, 40, and 50 year olds, respectively.

**Serum Lipid Response**

Basal serum lipid concentrations were not different between age or treatment groups (Table 2). For all ages combined, ingestion of DION resulted in 8% reductions in serum HDL-C during weeks 1-4 (P < 0.05; Treatment-by-Week effect). The observed decrease in serum HDL-C concentrations was not related to age (r²=0.001), but was significantly related to pre-supplementation HDL-C concentrations (r²=0.284). Serum low-density lipoprotein cholesterol (LDL-C) and total cholesterol (Total-C) concentrations were not altered by age or supplementation.

**Blood Chemistry Response**

Serum concentrations of gamma-glutamyltransferase, aspartate aminotransferase and alanine aminotransferase remained unchanged throughout the 4-week supplementation period
and were not affected by age (data not shown). There was also no age or supplement related change in serum concentrations of protein, albumin, or globulin (data not shown).

**Dietary Analysis**

There were no significant differences in dietary macronutrient intake throughout the study for either age or treatment group (Table 3). There were also no age or group differences observed for alcohol, saturated fat, monounsaturated fat, polyunsaturated fat, or cholesterol intake (data not shown). Subjects reported no use of prescription medications and only occasional use of over the counter medications such as analgesics.

**Discussion**

Two novel findings of the present research are 1) chronic DION ingestion increases serum free testosterone concentrations in 30-59 year old men and 2) the ingestion of saw palmetto in the presence of exogenous androgens does not prevent the 5α-reduction of ingested androgens. The present results also extend the previously observed findings in young men [10] that ingestion of DION does not prevent aromatization of ingested androstenedione or increase serum total testosterone concentrations in older men.

Although androstenedione can be enzymatically converted to testosterone [11-13], daily doses of 100-300 mg androstenedione do not chronically increase serum total testosterone concentrations in young [1-3] or older men [5,14]. The present results suggest that ingestion of DION, which contains androstenedione, also does not increase serum total testosterone concentrations in men between 30 and 59 years of age. In contrast, Leder et al. [3] observed that serum total testosterone concentrations increased ~34% following a single 300-mg androstenedione dose. These authors observed that serum total testosterone concentrations returned to baseline within ~4-6 hours, suggesting that daily doses of 300-mg
androstenedione or less are unlikely to produce chronic elevations in serum total testosterone concentrations.

Ingestion of androstenedione alone [1, 4], or formulated as DION [10], does not increase serum free testosterone concentrations in 19-24 year old men. In contrast, DION ingestion increases serum free testosterone concentrations in 30-59 year old men. The elevations in serum free testosterone concentrations in DION in the current study confirm our previous observations of elevated serum free testosterone concentrations with chronic androstenedione ingestion in older men [5]. Consistent with the previously observed age related declines in serum free testosterone concentrations [15-18], the serum free testosterone concentrations in the current older subjects were lower than in the younger subjects in our previous research [10], suggesting that age and basal serum free testosterone concentrations may affect the free testosterone response to androstenedione intake. In addition, the large degree of unexplained individual variability in the serum testosterone response to androstenedione ingestion in young men [3] suggests that other, unknown factor(s) may influence the testosterone response to androstenedione ingestion.

The use of weak androgens to increase serum testosterone concentrations is based on the findings that DHEA [19, 20] and androstenedione [19] ingestion increase serum testosterone concentrations in women. Since women have much lower serum testosterone concentrations than men, and since serum free testosterone concentrations decline in aging men [15-18], it has been hypothesized that DHEA ingestion may enhance serum free testosterone concentrations in older men. However, the ingestion of DHEA does not appear to increase serum free or total testosterone concentrations in men up to 70 years old [14, 20-23]. Therefore, it is likely that the increase in serum free testosterone concentrations in the
present study was a consequence of the conversion of androstenedione, rather than DHEA, to testosterone.

In spite of the inclusion of chrysin, indole-3-carbinol, saw palmetto, and Tribulus terrestris the hormonal response to DION ingestion did not differ from that observed with androstenedione [5] ingestion. While chrysin [7] inhibits the aromatization of androgens and indole-3-carbinol increases the hydroxylation and excretion of estrogens [6], these compounds do not appear to prevent the in vivo increase in serum estrogen concentrations after androstenedione ingestion. Although saw palmetto has been observed in vitro to impair the 5α reduction of endogenous androgens to DHT [8], the increase in serum DHT with DION ingestion is comparable to the increase observed with androstenedione ingestion alone [5], suggesting that saw palmetto does not reduce the in vivo conversion of ingested androgens to DHT. The serum testosterone response to DION [10, present results] is remarkably similar to previous observations made with only androstenedione ingestion [1,5], suggesting that the inclusion of Tribulus terrestris also does not alter the serum testosterone response to ingesting androstenedione. Taken together, these results suggest that the inclusion of indole-3-carbinol, chrysin, saw palmetto, and Tribulus terrestris in conjunction with androstenedione does not alter the hormonal response to androstenedione ingestion.

DION is designed to enhance serum testosterone concentrations and enhance the adaptations to resistance training. Exogenous testosterone administration resulting in large increases in serum free and total testosterone concentrations increases muscle strength and size in young [24] and older [25] men. However, the increases in serum testosterone concentrations typically required to increase muscle size and strength [25] are ~4-10 fold larger than the currently observed increases in serum free testosterone [24]. DION does not
enhance the adaptations to resistance training nor increase serum testosterone concentrations in young men [10]. Whether an anabolic effect is seen with the 38-45% increases in serum free testosterone concentrations associated with androstenedione alone [5] or formulated as DION [present results] in older men is unknown.

Physiologic levels of estradiol stimulate HDL-C synthesis in normal men [26], and elevated serum estrogens can have positive effects on vascular reactivity and serum lipid concentrations [27]. However, in spite of the elevated serum estradiol concentrations presently observed, serum HDL-C concentrations decreased with DION ingestion. Phillips et al. [28] observed that -20% elevations in serum estradiol concentrations in men are associated with the occurrence of myocardial infarction. There is also a risk for gynecomastia associated with elevated serum estrogen levels [29]. While it is unlikely that the increased serum estradiol concentrations in the present study had any long-term effects on health, the effects of increased serum estradiol levels subsequent to long term DION ingestion are unknown.

Ballantyne et al. [4] observed that androstenedione ingestion augments the increase in serum estrogens observed immediately after resistance exercise. However, in the current study blood samples were obtained at least 8-10 h after any physical activity and the increased serum estradiol concentrations in response to DION did not differ between physically active and inactive subjects. These data suggest that physical activity does not alter the chronic increase in serum estradiol concentrations associated with androstenedione ingestion.

Elevated serum androstenedione concentrations and a 150 % reduction of the serum testosterone to androstenedione ratio have been observed in patients with pancreatic cancer
The increase in serum androstenedione and 135% decrease in the testosterone to androstenedione ratio observed during ingestion of DION are suggestive of an elevated risk for pancreatic carcinoma.

The effect of weak androgen ingestion on prostate health is of particular concern in the aging male. We have previously observed that 300 mg·d⁻¹ androstenedione ingestion does not alter serum PSA concentrations in healthy men [5]. The present findings confirm these findings, and suggest that chrysin, indole-3-carbinol, saw palmetto and Tribulus terrestris also do not alter serum PSA concentrations. While serum PSA concentrations were not altered during the course of this study, 30% of males with prostatic tumors demonstrate normal serum PSA concentrations [31]. In addition, in dogs benign prostatic hypertrophy has been induced with ~100% increases in serum estradiol and DHT concentrations [32]. Therefore, the 103% increase in serum estradiol and 71% increase in serum DHT concentrations suggest that the potential risk of impaired prostate function with long term DION use should not be disregarded.

In conclusion, the ingestion of 300-mg·day⁻¹ androstenedione combined with herbal extracts containing aromatase and 5α-reductase inhibitors does not increase serum total testosterone or PSA concentrations in 30-59 year old men, but does increase serum concentrations of free testosterone, estradiol, androstenedione, and DHT. Thus, the addition of these herbal extracts to androgenic compounds does not appear to mitigate the serum hormonal and lipid response to androgen ingestion. The changes in serum hormone and lipid concentrations associated with androgen ingestion suggest possible negative health consequences with long-term use of these products.
References


Table and Figures

Table 1. Anthropometric data

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Data are Means ± SE.
Table 2. Serum cholesterol concentrations during four weeks of supplementation

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Data are Means ± SE.
Figure 1. Serum total testosterone concentrations during four weeks of nutritional supplementation.
Figure 2. Serum free testosterone concentrations during four weeks of nutritional supplementation.

* significantly different from week 0 for DION (Treatment-by-Week effect, P < 0.05); † 50 year olds significantly different from 30 year olds at baseline (Age-by-Week effect, P < 0.05).
Figure 3. Serum androstenedione concentrations during four weeks of nutritional supplementation.

*significantly different from week 0 for DION (Treatment-by-Week effect, P < 0.05); ‡ 30 year olds significantly different from 40 and 50 year olds at baseline (Age-by-Week effect, P < 0.05).
Figure 4. Serum dihydrotestosterone concentrations during four weeks of nutritional supplementation.

*significantly different from week 0 for DION (Treatment-by-Week effect, P < 0.05); ‡ 30 year olds significantly different from 40 and 50 year olds at baseline (Age-by-Week effect, P < 0.05).
Figure 5. Serum estradiol concentrations during four weeks of nutritional supplementation.

* significantly different from week 0 for DION (Treatment-by-Week effect, P < 0.05).
Figure 6. Serum prostate specific antigen concentrations during four weeks of nutritional supplementation.
CHAPTER 5. ENDOCRINE AND LIPID RESPONSES TO CHRONIC ANDROSTENEDIOL-HERBAL SUPPLEMENTATION IN 30-58 YEAR OLD MEN


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\textsuperscript{4}Major Professor, professor, and corresponding author

\textsuperscript{5}Contributed significantly to sample collection and overall research completion

Abstract

Objective. The effectiveness of an androgenic nutritional supplement designed to enhance serum testosterone concentrations and prevent the formation of dihydrotestosterone and estrogens was investigated in healthy 30 to 58 year old men. Design. Subjects were randomly assigned to consume a nutritional supplement (AND-HB) containing 300-mg androstenediol, 480-mg saw palmetto, 450-mg indole-3-carbinol, 300-mg chrysin, 1,500 mg gamma-linolenic acid, and 1,350-mg Tribulus terrestris per day (n=28), or placebo (n=27) for 28 days. Subjects were stratified into age groups to represent the fourth (30 yr olds, n=20),
fifth (40 yr olds, n=20) and sixth (50 yr olds, n=16) decades of life. **Measurements.** Serum free testosterone, total testosterone, androstenedione, dihydrotestosterone, estradiol, prostate specific antigen, and lipid concentrations were measured before supplementation and weekly for 4 weeks. **Results.** Basal serum total testosterone, estradiol, and prostate specific antigen (PSA) concentrations were not different between age groups. Basal serum free testosterone concentrations were higher (P<0.05) in the 30- (70.5 ± 3.6 pmol/L) than in the 50 year olds (50.8 ± 4.5 pmol/L). Basal serum androstenedione and dihydrotestosterone (DHT) concentrations were significantly higher in the 30- (for androstenedione and DHT respectively; 10.4 ± 0.6 nmol/L and 2198.2 ± 166.5 pmol/L) than in the 40- (6.8 ± 0.5 nmol/L and 1736.8 ± 152.0 pmol/L) or 50 year olds (6.0 ± 0.7 nmol/L and 1983.7 ± 147.8 pmol/L). Basal serum hormone concentrations did not differ between the treatment groups. Serum concentrations of total testosterone and PSA were unchanged by supplementation. Ingestion of AND-HB resulted in increased (P<0.05) serum androstenedione (174%), free testosterone (37%), DHT (57%), and estradiol (86%) throughout the 4 weeks. There was no relationship between the increases in serum free testosterone, androstenedione, DHT, or estradiol and age (r² = 0.08, 0.03, 0.05 and 0.02, respectively). Serum HDL-C concentrations were reduced (P<0.05) by 0.14 mmol·l⁻¹ in AND-HB. **Conclusions.** These data indicate that ingestion of androstenediol combined with herbal products does not prevent the formation of estradiol and dihydrotestosterone.

**Introduction**

Androstenediol (4-androstene-3ß, 17ß-diol) is an androgenic precursor to testosterone [1,2]. Advertisements claim that ingesting androstenediol increases serum testosterone concentrations and augments the gains in muscle mass and strength associated with
resistance training. However, in men, serum testosterone concentrations are not increased within 90 minutes of a single 200 mg dose [3] or when blood is sampled ~12 h after ingesting 100 mg [4] androstenediol but serum estradiol concentrations are increased ~12 h after intake.

Several herbal extracts have been demonstrated to alter steroid metabolism and may change the endocrine response to androstenediol intake. Saw palmetto [5] and gamma linolenic acid (γLA) [6] inhibit the in vitro 5α reduction of androgens to dihydrotestosterone (DHT). Chrysin has been shown to impair aromatization in vitro [7] and indole-3-carbinol has been shown to enhance the urinary clearance of estrogens in vivo [8]. Tribulus terrestris is purported to increase testosterone concentrations by increasing serum luteinizing hormone concentrations, although this claim has not been verified. While androstenediol ingestion alone does not appear to alter serum testosterone concentrations [3,4], inclusion of these herbal extracts with androstenediol may prevent the breakdown of androstenediol to estrogens or dihydrotestosterone resulting in increased serum testosterone concentrations. Therefore, the purpose of this project was to determine whether chronic ingestion of a nutritional supplement (AND-HB) that combines androstenediol, saw palmetto, γLA, indole-3-carbinol, chrysin, and Tribulus terrestris increases serum testosterone concentrations while preventing increased serum estradiol and DHT concentrations in healthy men.

Methods

Subjects and General Design

Fifty-six healthy men (30 - 58 y) ingested either a rice flour placebo (PL) or AND-HB for four weeks. Blood samples were collected each week for the measurement of serum hormone concentrations and blood chemistry. Prior to participating in this project all
subjects provided informed consent, were questioned to ensure they were not currently using nutritional supplements, and completed a written medical history to eliminate subjects with a known chronic disease. The human subjects review committee at Iowa State University provided approval for this project.

**Supplementation**

Subjects were randomly assigned in a double blind, counter balanced manner to consume unmarked capsules containing either PL or AND-HB. Subjects were instructed to consume the supplements in equal doses t.i.d. (before 0900 h, at 1500 h, and before bedtime). AND-HB contained daily doses of 300 mg androstenediol, 480 mg saw palmetto, 450 mg indole-3-carbinol, 300 mg chrysin, 1,500 mg γLA, and 1,350 mg Tribulus terrestris. The capsules were provided by a supplier of nutritional supplements (Experimental and Applied Sciences, Golden, CO) and were assayed for content and purity (~99%) using high-performance liquid chromatography (HPL-C) by an independent laboratory (Integrated Biomolecule, Tucson, AZ). Subject compliance to the supplementation regime was assessed through written records and the return of supplement containers at the conclusion of the study.

**Diet and Activity**

Subjects were instructed to maintain their normal diet and activity pattern throughout the course of the study and also record their diet and activity for the 2 d prior to each blood sampling. Dietary composition was analyzed using commercial software (Nutritionist 4, N-Squared computing, San Bruno, CA).
Blood Sample Collection and Analysis

Fasting blood samples were collected between 0630 and 0800 at baseline and once per week, ~8-10 hours after supplement ingestion, on the same day each week, for 28 days. Blood samples (~20 ml) were collected without stasis from an antecubital vein and immediately placed into an ice bath until centrifugation and serum separation. Blood samples were analyzed for liver function enzyme, lipid, and protein composition by a commercial laboratory (Quest Diagnostics, Inc., Wood Dale, IL). Serum concentrations of total testosterone, free testosterone, androstenedione, and estradiol were measured via commercial tracer analog radioimmunoassay kits (Diagnostic Products Corp, Los Angeles, CA; and Diagnostic Systems Laboratory, Webster, TX). Serum DHT and prostate specific antigen (PSA) concentrations were measured with commercially available enzyme-linked immunosorbent assay (ELISA; Bio-Clin, Inc, St. Louis, MO; Immuno-Biological Laboratories, Hamburg, Germany). The samples for each subject were analyzed in duplicate within the same assay and the intra-assay coefficients of variation for total testosterone, free testosterone, estradiol, androstenedione, DHT and PSA were 6.1%, 7.2%, 6.6%, 5.8%, 3.2% and 6.6%, respectively. According to the manufacturers of the RIA and ELISA kits, there is no detectable cross reactivity of the assays for androstenediol, androstenedione, DHT, estradiol or testosterone.

Calculations and Statistics

To facilitate data analysis and presentation, subjects were grouped by age to represent the fourth (30 yr olds, n=20), fifth (40 yr olds, n=20) and sixth (50 yr olds, n=16) decades of life. Statistical analyses of the data were performed using a 3 factor (Week by Treatment by Age group) repeated measures analysis of variance (ANOVA) with commercial software.
(SPSS Inc, Chicago, IL). Specific mean differences (P < 0.05) were identified using Student Newman-Keuls post hoc comparisons. Because basal serum hormone concentrations may alter the endocrine response to androstenediol supplementation, analysis of covariance (ANCOVA) was performed using basal hormone concentrations as the covariate. Relationships between the effects of supplementation and age were analyzed using simple linear regression. The percent change in serum analytes was calculated as the mean change from baseline for all subjects during weeks 1-4. Data are presented throughout as means ± SE.

Results

Subjects

Subjects came from a wide variety of occupations and physical activity levels. No changes in physical activity patterns were reported during the course of the investigation. Twelve subjects in AND-HB and 10 in PL reported regular participation in aerobic activity such as running and basketball while resistance exercise was reported in 4 subjects in AND-HB and 6 in PL. No differences or changes in height, body mass, or body mass index (BMI) were found for any treatment or age group (Table 1). One 50-year-old subject in the placebo group was diagnosed with non-insulin dependent diabetes mellitus during the study, informed of the condition, and his data have been excluded from all analysis. This project was part of a larger study, and the data for the placebo group has been reported elsewhere [9,10].
Hormonal Response to Supplementation

The results of the ANCOVA revealed no effect of basal serum hormone concentrations on the hormonal response to AND-HB. Therefore, actual, rather than adjusted means from the ANCOVAs are presented throughout the text.

Serum total testosterone concentrations were not different between age or treatment groups at baseline, nor were they altered by supplementation (Figure 1).

Basal serum free testosterone concentrations were significantly related to age ($r^2=0.23$), and were lower in the 50 year olds than the 30 year olds (Figure 2; $P<0.05$). For all ages combined, ingestion of AND-HB resulted in a mean increase ($P<0.05$) of 37% in serum free testosterone concentrations during weeks 1-4. There was no relationship between the change in serum free testosterone concentrations and age ($r^2=0.08$).

Basal serum androstenedione concentrations were higher in the 30 year olds ($P < 0.05$) than in the 40 or 50 year olds (Figure 3). For all ages combined ingestion of AND-HB resulted in a 174% increase ($P < 0.05$) in serum androstenedione concentrations during weeks 1-4. The increase in serum androstenedione concentrations was not related to age ($r^2=0.029$).

Basal serum DHT concentrations were higher in the 30 year olds than in the 40 or 50 year olds ($P<0.05$; Figure 4). For all ages combined, ingestion of AND-HB resulted in a 57% increase ($P < 0.05$) in serum DHT concentrations during week 1-4. The increase in serum DHT concentration was not related to age ($r^2=0.033$).

Basal serum estradiol concentrations were not different between age or treatment groups (Figure 5). For all ages combined, ingestion of AND-HB resulted in an 86% increase ($P < 0.05$) in serum estradiol concentrations during weeks 1-4. There was no relationship
between the mean increase in serum estradiol concentrations and age ($r^2=0.029$) or body mass index ($r^2=0.024$).

Age did not influence the changes in the testosterone to estradiol ratio. The ratio of total testosterone to estradiol for all ages combined decreased ($P<0.05$) during weeks 1-4 in AND-HB ($109 \pm 10$ vs. $65 \pm 5$) and remained stable in PL ($106 \pm 9$ vs. $113 \pm 9$). Similarly, for all ages combined, the ratio of free testosterone (pmol·l⁻¹) to estradiol (pmol·l⁻¹) decreased ($P<0.05$) during weeks 1-4 in AND-HB ($0.37 \pm 0.04$ vs. $0.28 \pm 0.02$) and did not change in PL ($0.37 \pm 0.03$ vs. $0.39 \pm 0.03$).

Serum PSA concentrations were not affected by age or supplementation (Figure 6). The mean serum PSA concentrations throughout the 4 weeks of supplementation for all treatment groups combined were $2.1$ ng·ml⁻¹, $2.6$ ng·ml⁻¹ and $2.5$ ng·ml⁻¹ for the 30, 40, and 50 year olds, respectively.

**Serum Lipid Response**

For all ages combined, ingestion of AND-HB resulted in an 11% decrease ($P < 0.05$) in serum HDL-C concentration during weeks 1-4 (Table 2). The decrease in serum HDL-C concentrations was independent of age ($r^2=0.001$) but was positively related to pre-supplementation HDL-C concentrations ($r^2=0.284$). Serum low-density lipoprotein cholesterol (LDL-C) and total cholesterol (Total-C) concentrations were not altered by age or treatment.

**Blood Chemistry Response**

Serum concentrations of gamma-glutamyltransferase, aspartate aminotransferase, and alanine aminotransferase remained unchanged throughout the 4-week supplementation period.
and were not affected by age. There was also no age or supplement related change in serum concentrations of protein, albumin, or globulin (data not shown).

Dietary Analysis

There were no age or treatment differences in dietary macronutrient intake (Table 3). There were also no age or treatment differences in saturated fat, monounsaturated fat, polyunsaturated fat, or cholesterol intake (Table 4).

Discussion

The current results demonstrate that chronic ingestion of 100 mg androstenediol taken t.i.d. does not increase serum total testosterone concentrations measured ~8-10 h after intake. In addition, the use of herbal aromatase and 5α-reductase inhibitors does not prevent conversion of the ingested androstenediol to estradiol and DHT. However, unlike previous observations with chronic 100 mg b.i.d. androstenediol ingestion [4], chronic AND-HB ingestion elevates serum free testosterone concentrations.

The ingestion of weak androgens, such as androstenedione and androstenediol, is claimed by nutritional supplement marketers to increase serum testosterone concentrations. Although comparing results across studies and subject populations should be done with caution, serum total testosterone concentrations are unchanged within a few hours of ingesting 100-200 mg androstenedione [3,11,12,13] or 8-12 h after ingesting 100-300 mg [4, 9,11,12,13] androstenedione. The addition of herbal extracts does not change the testosterone response to androstenedione ingestion within 6 h of intake [11,12]. In vitro, androstenediol is converted to testosterone ~3 times more readily than androstenedione [2], suggesting that androstenediol ingestion would more effectively increase serum testosterone concentrations than androstenedione. In spite of the greater in vitro conversion of
androstenediol to testosterone, serum testosterone concentrations are not changed with 90 min of 200 mg androstenediol intake [3] or at 12 h after 100 mg [4]. The current results indicate that the ingestion of herbal extracts does not change the serum total testosterone response to androstenediol.

Although oral intake of 200 mg androstenediol does not increase serum total testosterone concentrations acutely [3], and intake of 100 mg androstenediol does not increase serum testosterone after ~8 h (present study), we have recently observed that sublingual intake of 20 mg androstenediol causes an acute ~75% increase in serum total testosterone concentrations lasting for at least 3 hours [14]. It therefore appears that while androstenediol can be converted to testosterone, the processes of digestion and hepatic catabolism destroy much of the ingested androstenediol.

Serum free testosterone concentrations are unchanged after ingestion of a single dose of 200 mg androstenediol [3] or chronic ingestion of 100 mg b.i.d. [4]. The increase in serum free testosterone concentrations observed in the present study may be due to the higher daily dose of androstenediol administered. Since we have previously observed that herbal extracts do not alter the hormonal response to androstenedione ingestion [10,12], it is unlikely that the increased serum free testosterone concentrations resulted from the addition of saw palmetto, γLA, indole-3-carbinol, chrysin, and Tribulus terrestris.

Increased serum free testosterone in conjunction with unchanged total testosterone, protein, and albumin concentrations suggests that androstenediol ingestion may exert an affect on sex hormone binding globulin (SHBG). Since SHBG binds more favorably to DHT than testosterone [15], it is possible that androstenediol ingestion causes a shift in the binding of SHBG from testosterone to DHT resulting in increased serum free testosterone.
concentrations. It is also possible that, similar to androstenedione ingestion [13], androstenediol ingestion results in reduced serum SHBG concentrations. Since we were unable to measure SHBG in the current study, the effect of androstenediol ingestion on SHBG remains unknown.

The tracer analog method for measuring free testosterone has been criticized based on findings of a positive relationship between SHBG and measured free testosterone concentrations, and the suggestion that this method detects a constant fraction of the total testosterone concentrations, rather than the testosterone that is free from binding proteins [16]. The increased serum free testosterone concentrations with no concomitant increase in total testosterone concentrations in the present study suggests that the tracer analog method does not measure a constant fraction of serum total testosterone. This finding supports the work of others [17,18] demonstrating that this method accurately measures free testosterone concentrations, and suggests that the observed increased serum free testosterone concentrations are not an artifact of the method of measurement.

Marketing claims purport that androstenediol ingestion enhances the effects of resistance training. On the contrary, Broeder et al. [4] found that androstenediol ingestion does not increase serum free testosterone concentrations or enhance muscle mass or strength gains associated with resistance training in men. Previous observations indicate that larger (~5 fold) increases in serum testosterone concentrations increase muscle protein synthesis and the adaptations to resistance training [19,20,21]. Since we did not assess muscle mass or strength, the effects of the 37% increase in serum free testosterone concentrations on muscle mass remain unknown.
The action of 5α reductase converts androgens into DHT [22], which has been associated with benign prostate hypertrophy [23]. In spite of the inclusion of γLA and saw palmetto, which inhibit 5α reductase in vitro [5,6], AND-HB ingestion results in increased serum DHT concentrations. Since the serum DHT response to androstenediol intake has not been previously examined, it is difficult to determine whether the inclusion of γLA and saw palmetto in AND-HB altered the serum DHT response. However, the increases in serum DHT concentrations with AND-HB intake were similar in magnitude to our previous observations with androstenedione [9]. Therefore, it appears that γLA and saw palmetto do not effectively inhibit the conversion of exogenous androgens into DHT.

Some marketers of nutritional supplements claim that androstenediol does not undergo aromatization. However, elevated serum estradiol concentrations in conjunction with androstenediol intake have been reported [4,14], suggesting that androstenediol may undergo aromatization. Furthermore, the elevations in serum estradiol after ingestion of 100 mg androstenediol t.i.d. are very similar in magnitude to those found after ingestion of 100 mg androstenedione t.i.d. ingestion in a similar population [9,10] suggesting that androstenediol is aromatized to the same extent as androstenedione.

In vitro, chrysin inhibits aromatase activity [7]. In vivo, indole-3-carbinol inhibits aromatization and enhances estrogen clearance [8]. However, the inclusion of these herbal extracts in AND-HB did not prevent elevated serum estradiol concentrations. Since Broeder et al. [4], used 200 mg/day androstenediol and the present study used 300 mg/day, and the increase in serum estradiol was of a larger magnitude in the present study, indole-3-carbinol
and chrysin do not appear to be effective at inhibiting aromatization in the presence of exogenous androgens.

Similar to our previous observations with androstenedione ingestion [9,10], short-term androstenediol ingestion does not alter serum PSA concentrations. Although this indicates that short-term androstenediol ingestion does not increase the risk of prostate cancer, the development of prostate cancer occurs slowly and is not always detectable with measurements of serum PSA [24].

The ingestion of androstenediol is purported to enhance health and vitality. However, the health related consequences of long-term androstenediol ingestion have not been examined and the hormonal milieu associated with chronic androstenedione ingestion may increase the risk for disease. The testosterone/androstenedione ratio is lower in patients with pancreatic cancer [25]. In the present study, the testosterone/androstenedione ratio after AND-HB intake was one half of that found in patients with pancreatic cancer [25], suggesting that AND-HB intake may predispose users to pancreatic cancer. A 20% increase in serum estradiol concentrations has been observed in men with myocardial infarction [26], and increased serum estradiol in conjunction with increased serum DHT has been experimentally demonstrated to induce benign prostate hyperplasia [23]. Furthermore, since relatively small (i.e., 1 nmol/L) increases in serum androstenedione are associated with a significant increases in the risk for prostate cancer, the ~12 nmol/L increase in serum androstenedione observed with AND-HB intake may be of clinical relevance. The reduced serum HDL-C concentrations associated with androstenediol ingestion are indicative of an ~12% increase in the risk for heart disease [27]. Although none of these conditions were observed during the present study, and it is unlikely that these chronic diseases would be
detected during the course of 4 weeks, untoward health consequences with long-term androstenediol intake cannot be ruled out.

Conclusion

Chronic ingestion of 100 mg androstenediol t.i.d. produces modest increases in serum free testosterone, and more pronounced increases in serum estradiol, androstenedione, and DHT concentrations while reducing serum HDL-C concentrations in 30-58 year old men. The changes in the serum hormonal milieu were observed in spite of the inclusion of saw palmetto, γLA, indole-3-carbinol, chrysin, and Tribulus terrestris suggesting that, in the doses given, these herbal extracts do not prevent the aromatization or 5α reduction of ingested androstenediol. Although the health related effects of long-term androstenediol ingestion are unknown, the observed changes in the serum hormone profile suggest that caution is advisable in its use as a nutritional supplement.

References


**Table and Figures**

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<td>96±6</td>
<td>85±5</td>
<td>101±10</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>29±2</td>
<td>27±2</td>
<td>30±2</td>
</tr>
</tbody>
</table>

Data are means ± SE.
Table 2. Serum cholesterol concentrations during four weeks of supplementation

<table>
<thead>
<tr>
<th>Week (n)</th>
<th>30's PL (n=10)</th>
<th>30's AND-HB (n=10)</th>
<th>40's PL (n=10)</th>
<th>40's AND-HB (n=10)</th>
<th>50's PL (n=7)</th>
<th>50's AND-HB (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HDL-C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.95±0.06</td>
<td>1.09±0.08</td>
<td>0.98±0.11</td>
<td>1.09±0.06</td>
<td>1.23±0.13</td>
<td>1.09±0.08</td>
</tr>
<tr>
<td>1</td>
<td>0.94±0.06</td>
<td>0.97±0.06*</td>
<td>0.99±0.10</td>
<td>0.96±0.07*</td>
<td>1.21±0.13</td>
<td>1.00±0.09*</td>
</tr>
<tr>
<td>2</td>
<td>0.90±0.06</td>
<td>0.94±0.07*</td>
<td>0.99±0.09</td>
<td>0.89±0.05*</td>
<td>1.22±0.13</td>
<td>0.93±0.08*</td>
</tr>
<tr>
<td>3</td>
<td>0.93±0.07</td>
<td>0.96±0.05*</td>
<td>1.00±0.09</td>
<td>0.92±0.04*</td>
<td>1.27±0.13</td>
<td>0.97±0.09*</td>
</tr>
<tr>
<td>4</td>
<td>0.96±0.07</td>
<td>0.95±0.06*</td>
<td>0.99±0.10</td>
<td>0.93±0.05*</td>
<td>1.21±0.13</td>
<td>0.95±0.11*</td>
</tr>
<tr>
<td><strong>LDL-C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3.0±0.2</td>
<td>3.0±0.3</td>
<td>3.1±0.3</td>
<td>3.0±0.2</td>
<td>3.3±0.3</td>
<td>3.1±0.2</td>
</tr>
<tr>
<td>1</td>
<td>2.8±0.3</td>
<td>3.1±0.2</td>
<td>3.4±0.3</td>
<td>2.9±0.1</td>
<td>3.4±0.4</td>
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</tr>
<tr>
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<td>3.5±0.5</td>
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<td>3.5±0.3</td>
<td>2.9±0.1</td>
</tr>
<tr>
<td>3</td>
<td>2.7±0.3</td>
<td>3.2±0.3</td>
<td>3.4±0.4</td>
<td>2.9±0.2</td>
<td>3.7±0.3</td>
<td>2.9±0.2</td>
</tr>
<tr>
<td>4</td>
<td>2.8±0.2</td>
<td>2.9±0.3</td>
<td>3.1±0.3</td>
<td>3.0±0.2</td>
<td>3.6±0.2</td>
<td>2.9±0.2</td>
</tr>
<tr>
<td><strong>Total-C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4.3±0.5</td>
<td>4.7±0.4</td>
<td>4.9±0.4</td>
<td>4.8±0.2</td>
<td>5.4±0.3</td>
<td>4.68±0.3</td>
</tr>
<tr>
<td>1</td>
<td>4.6±0.23</td>
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<td>5.5±0.4</td>
<td>4.6±0.6</td>
</tr>
<tr>
<td>2</td>
<td>4.7±0.3</td>
<td>4.9±0.3</td>
<td>5.2±0.5</td>
<td>4.7±0.2</td>
<td>5.5±0.4</td>
<td>4.6±0.5</td>
</tr>
<tr>
<td>3</td>
<td>4.4±0.3</td>
<td>4.9±0.3</td>
<td>5.0±0.4</td>
<td>4.5±0.2</td>
<td>5.7±0.4</td>
<td>4.5±0.6</td>
</tr>
<tr>
<td>4</td>
<td>4.5±0.3</td>
<td>4.8±0.3</td>
<td>4.9±0.4</td>
<td>4.7±0.2</td>
<td>5.5±0.2</td>
<td>4.6±0.6</td>
</tr>
</tbody>
</table>

Data are means ± SE. Units are mmol/L. * Significantly different from week 0 for AND-HB (Treatment-by-Week effect, P < 0.05)
Table 3. Dietary macronutrient intake during four weeks of supplementation

<table>
<thead>
<tr>
<th>Week</th>
<th>30's PL (n=10)</th>
<th>AND-HB (n=10)</th>
<th>40's PL (n=10)</th>
<th>AND-HB (n=10)</th>
<th>50's PL (n=7)</th>
<th>AND-HB (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KJ $\cdot 10^3$</td>
<td>0</td>
<td>10.0±0.7</td>
<td>9.7±0.9</td>
<td>9.6±0.8</td>
<td>7.8±1.1</td>
<td>8.5±1.3</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>9.9±1.0</td>
<td>10.1±0.7</td>
<td>10.0±1.2</td>
<td>9.3±0.7</td>
<td>8.7±1.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10.7±0.8</td>
<td>9.5±0.8</td>
<td>10.8±1.1</td>
<td>9.7±0.6</td>
<td>8.8±0.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>9.9±0.8</td>
<td>9.7±0.6</td>
<td>8.8±1.1</td>
<td>9.9±0.8</td>
<td>8.6±0.7</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>9.8±0.7</td>
<td>11.1±0.9</td>
<td>9.8±0.9</td>
<td>9.2±0.7</td>
<td>7.1±1.0</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>0</td>
<td>331±29</td>
<td>346±30</td>
<td>330±29</td>
<td>287±25</td>
<td>286±71</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>339±42</td>
<td>344±36</td>
<td>338±47</td>
<td>330±31</td>
<td>296±42</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>366±34</td>
<td>346±34</td>
<td>322±34</td>
<td>318±31</td>
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<td>346±21</td>
<td>364±36</td>
<td>290±43</td>
<td>323±35</td>
<td>288±37</td>
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<td>4</td>
<td>340±26</td>
<td>383±42</td>
<td>348±40</td>
<td>301±34</td>
<td>217±17</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0</td>
<td>78±8</td>
<td>58±11</td>
<td>68±10</td>
<td>71±6</td>
<td>51±9</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>77±10</td>
<td>68±6</td>
<td>72±10</td>
<td>64±8</td>
<td>58±12</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>83±10</td>
<td>61±9</td>
<td>93±16</td>
<td>70±5</td>
<td>59±5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>70±12</td>
<td>57±7</td>
<td>62±6</td>
<td>73±9</td>
<td>60±6</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>76±12</td>
<td>71±8</td>
<td>68±10</td>
<td>67±5</td>
<td>56±12</td>
</tr>
</tbody>
</table>

Data are means ± SE.
Table 4. Dietary fat and cholesterol intake during four weeks of supplementation

<table>
<thead>
<tr>
<th>Week</th>
<th>PL (n=10)</th>
<th>AND-HB (n=10)</th>
<th>PL (n=10)</th>
<th>AND-HB (n=10)</th>
<th>PL (n=7)</th>
<th>AND-HB (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sat (g) 0</td>
<td>25.9±3.5</td>
<td>21.7±5.0</td>
<td>20.8±3.9</td>
<td>24.5±2.6</td>
<td>15.3±3.5</td>
<td>22.1±3.0</td>
</tr>
<tr>
<td>1</td>
<td>24.1±2.8</td>
<td>24.6±3.4</td>
<td>24.0±3.6</td>
<td>20.1±2.4</td>
<td>16.9±2.4</td>
<td>26.8±2.6</td>
</tr>
<tr>
<td>2</td>
<td>27.9±3.5</td>
<td>21.5±4.6</td>
<td>29.7±6.3</td>
<td>23.2±3.1</td>
<td>20.2±3.0</td>
<td>21.8±3.1</td>
</tr>
<tr>
<td>3</td>
<td>23.2±4.1</td>
<td>20.8±2.7</td>
<td>20.0±2.5</td>
<td>22.7±2.5</td>
<td>18.9±2.2</td>
<td>18.3±3.6</td>
</tr>
<tr>
<td>4</td>
<td>26.1±4.9</td>
<td>23.3±3.7</td>
<td>19.0±2.2</td>
<td>23.8±1.9</td>
<td>18.6±4.6</td>
<td>23.1±3.4</td>
</tr>
<tr>
<td>Mon (g) 0</td>
<td>23.4±2.8</td>
<td>18.0±3.4</td>
<td>17.9±2.0</td>
<td>21.8±1.9</td>
<td>17.8±4.4</td>
<td>19.5±2.6</td>
</tr>
<tr>
<td>1</td>
<td>23.5±5.0</td>
<td>24.8±2.4</td>
<td>24.8±3.6</td>
<td>19.4±2.7</td>
<td>19.7±6.0</td>
<td>32.2±2.5</td>
</tr>
<tr>
<td>2</td>
<td>26.2±4.9</td>
<td>18.6±2.7</td>
<td>33.1±6.4</td>
<td>23.2±2.5</td>
<td>18.4±2.0</td>
<td>23.8±3.8</td>
</tr>
<tr>
<td>3</td>
<td>20.7±3.9</td>
<td>17.7±2.7</td>
<td>18.5±3.1</td>
<td>24.2±4.2</td>
<td>17.8±2.6</td>
<td>21.0±3.8</td>
</tr>
<tr>
<td>4</td>
<td>20.8±3.1</td>
<td>26.1±3.4</td>
<td>22.7±3.0</td>
<td>20.5±2.5</td>
<td>16.5±4.7</td>
<td>18.1±3.0</td>
</tr>
<tr>
<td>Chl (mg) 0</td>
<td>311±60</td>
<td>246±48</td>
<td>264±43</td>
<td>330±52</td>
<td>247±48</td>
<td>259±75</td>
</tr>
<tr>
<td>1</td>
<td>245±52</td>
<td>273±54</td>
<td>193±34</td>
<td>248±45</td>
<td>155±32</td>
<td>366±70</td>
</tr>
<tr>
<td>2</td>
<td>265±59</td>
<td>184±30</td>
<td>327±82</td>
<td>275±45</td>
<td>251±64</td>
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<tr>
<td>3</td>
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<td>366±165</td>
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<td>204±38</td>
<td>290±57</td>
<td>200±26</td>
<td>337±68</td>
<td>158±39</td>
<td>289±84</td>
</tr>
</tbody>
</table>

Data are means ± SE. Sat = saturated fat, Mon = monounsaturated fat, Chl = cholesterol
Figure 1. Serum total testosterone concentrations during four weeks of nutritional supplementation.

Data are means ± SE.
Figure 2. Serum free testosterone concentrations during four weeks of nutritional supplementation.

* Significantly different from week 0 for AND-HB (Treatment by Week effect, P < 0.05); † 50 year olds significantly different from 30 year olds at baseline (Age by Week effect, P < 0.05). Data are means ± SE.
Figure 3. Serum androstenedione concentrations during four weeks of nutritional supplementation.

* Significantly different from week 0 for AND-HB (Treatment by Week effect, $P < 0.05$); † 30 year olds significantly different from 40 and 50 year olds at baseline (Age by Week effect, $P < 0.05$). Data are means ± SE.
Figure 4. Serum dihydrotestosterone concentrations during four weeks of nutritional supplementation.

* Significantly different from week 0 for AND-HB (Treatment by Week effect, $P < 0.05$); † 30 year olds significantly different from 40 and 50 year olds at baseline (Age by Week effect, $P < 0.05$). Data are means ± SE.
Figure 5. Serum estradiol concentrations during four weeks of nutritional supplementation.

* Significantly different from week 0 for AND-HB (Treatment by Week effect, $P < 0.05$).

Data are means ± SE.
Figure 6. Serum prostate specific antigen concentrations during four weeks of nutritional supplementation.

Data are means ± SE.
CHAPTER 6. ACUTE HORMONAL RESPONSE TO SUBLINGUAL ANDROSTENEDIOL INTAKE IN YOUNG MEN

A paper published in the Journal of Applied Physiology

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\textsuperscript{2}Human Performance Laboratory, South Dakota State University Department of HPER, Brookings, SD
\textsuperscript{3}Graduate student, primary researcher and author.
\textsuperscript{4}Major Professor, professor, and corresponding author
\textsuperscript{5}Contributed significantly to sample collection and overall research completion

Abstract

The effectiveness of orally ingested androstenediol in raising serum testosterone concentrations may be limited due to hepatic breakdown of the ingested androgens. Since 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 males experienced in strength training (22.9 ± 1.2 y) consumed either 20 mg androstenediol in a sublingual cyclodextrin tablet (SL-DIOL) or placebo (PL) separated by at least one week in a randomized, double blind cross over manner. Blood samples were collected before supplementation and at 30-min intervals
for 3 h following supplementation. Serum hormone concentrations did not change in PL. Serum androstenedione concentrations were increased (P<0.05) above baseline (11.2 ± 1.1 nmol·L⁻¹) in SL-DIOL from 60-180 min after intake, and reached a peak concentration of 25.2 ± 2.9 nmol·L⁻¹ at 120 min. Serum free testosterone concentrations were increased from 86.2 ± 9.1 pmol·L⁻¹ in SL-DIOL from 30 to 180 min, and reached a peak concentration of 175.4 ± 12.2 pmol·L⁻¹ at 60 min. Serum total testosterone concentrations increased above basal (25.6 ± 2.3 nmol·L⁻¹) from 30-180 min in SL-DIOL, and reached a peak concentration of 47.9 ± 2.9 nmol·L⁻¹ at 60 min. Serum estradiol concentrations were elevated (P<0.05) above baseline (0.08 ± 0.01 nmol·L⁻¹) from 30-180 min in SL-DIOL and reached 0.14 ± 0.02 nmol·L⁻¹ at 180 min. These data indicate that sublingual cyclodextrin androstenediol intake increases serum androstenedione, free testosterone, total testosterone, and estradiol concentrations.

**Introduction**

The intake 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. While ~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 extrasplanchnic 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) do 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 (DHEA) (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, which 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 ~5 fold increases in serum testosterone concentrations in men (24,26,28). Since 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.

Methods

Approach to the Problem

Although changes in serum hormones in men are observed within 2 hours after ingesting a weak androgen, the lack of meaningful increases in serum testosterone concentrations suggests a large hepatic catabolism of the weak androgens. Since sublingual delivery avoids first pass clearance and cyclodextrins facilitate diffusion of an androgen across the oral mucosa, the presence or lack of effectiveness of sublingual cyclodextrin
androstenediol in increasing serum testosterone as well as other hormones should be evident within 3 hours of intake.

**Subjects**

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

**Blood Sample Collections**

Following an overnight fast, subjects reported to the laboratory between 5:30 and 7:00 a.m. After insertion of a flexible catheter into an antecubital vein, blood samples were collected without stasis from sitting subjects 20 min before supplementation, immediately prior to 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 three days prior to the initial trial and repeat the same diet and exercise protocol for the subsequent trial. Dietary records were analyzed 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, cross over 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™ Inc, Los Angeles, CA) was analyzed for hormone content using HPLC (Integrated
Bio-molecule Inc., Tucson, AZ) and each tablet contained 21.4 mg androstenediol, 3.7 mg
androstenedione, and no other steroid hormones.

**Body Composition**

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

**Hormonal Analysis**

Serum concentrations of total and free testosterone, androstenedione, and estradiol
were measured in duplicate via commercial tracer analog radioimmunoassay (RIA; Coat-A-
Count, Diagnostic Products Corp, 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 manufacturers 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 less than 0.5%, except for a 1.49% cross reactivity of the androstenedione assay for testosterone.

Calculations and Statistics

Data were analyzed using commercial software (SPSS Inc, Chicago IL). Statistical analyses were performed using a 2 factor (supplement by time) repeated measures analysis of variance (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 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 years. Subjects participated in resistance training 3.6 ± 0.5 days per week for 1.6 ± 0.2 h per exercise session. Five subjects reported regular participation in aerobic exercise 3.0 ± 0.5 days per weeks for 20 ± 3.2 min per 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 2 subjects reported previous use of nutritional supplements (creatine n=2, “weight gain powder” n=1) and no subject consumed any supplements during the 9 months prior to 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·d⁻¹ for three days prior to the initial trial, and repeated the same diet for the subsequent trial. Daily dietary intake was 161 ± 60 g·d⁻¹ (26 ± 6%) protein, 268 ± 49 g·d⁻¹ (46 ± 6%) carbohydrate, and 68 ± 9 g·d⁻¹ (37 ± 3%) fat.

**Hormonal Response**

Serum hormone concentrations did not change in PL. Serum androstenedione concentrations were increased from 60 - 180 min in SL-DIOL (Figure 1; P<0.05) while peak concentrations of 24.3 ± 1.5 nmol·L⁻¹ 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·min) compared with PL (96.0 ± 50.0 nmol/L·min).

SL-DIOL increased serum free testosterone concentrations (Figure 2; P<0.05) from 30 - 180 min after administration while peak concentrations (175.4 ± 2.2 pmol·L⁻¹) 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·min) compared with PL (750 ± 218 pmol/L·min). Serum free testosterone concentrations increased in all subjects in response to SL-DIOL.

SL-DIOL intake increased serum total testosterone concentrations (P<0.05; Figure 3) above basal (25.6 ± 2.3 nmol·L⁻¹) from 30-180 min and peak serum total testosterone concentrations were observed at 60 min (47.9 ± 2.9 nmol·L⁻¹). The AUC for total testosterone was higher (P<0.05) in SL-DIOL (2,673 ± 577 nmol/L·min) compared with PL.
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⁻¹) 30 - 180 min after administration in SL-DIOL (P<0.05; Figure 4) and reached 0.14 ± 0.02 nmol·L⁻¹ 180 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·min) compared with PL (1.12 ± 0.44 nmol/L·min).

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. Since a single 200 mg dose of androstenediol taken orally does not change serum free or total testosterone concentrations in young men (11), the current 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). Since 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, while 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), while SL-DIOL results in peak serum
testosterone concentrations ~ 60 min after intake. In hypogonadal men, 2.5-10 mg
cyclodextrin testosterone administered sublingually causes serum testosterone concentrations
to reach supranormal levels (~35-85 nmol·L⁻¹) 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). Since serum testosterone concentrations after SL-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,
allowing for the 30-40 min delay in the testosterone response following SL-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 sublingual cyclodextrin androstenediol 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), SL-DIOL increased serum testosterone concentrations. It is currently unknown if a
transient increase in serum testosterone of the magnitude elicited by SL-DIOL intake alters muscle mass or strength.

The tracer analog method for measuring free testosterone has been criticized recently based on findings of a positive relationship between SHBG 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 SL-DIOL intake suggests that androstenediol taken sublingually is converted to testosterone.

As previously observed with sublingual cyclodextrin testosterone intake (26,28), sublingual cyclodextrin androstenediol 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 (6,7,8,17,19,25) 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. Based upon the findings with ingested androstenedione (6,7,8,17,19,25), and sublingual cyclodextrin testosterone (26,28), the increased serum estradiol concentrations associated with SL-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 (unpublished
observations). These findings may be clinically significant, since 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 SL-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 d 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 DHEA (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 SL-DIOL produces a uniform hormonal response in young strength trained men; the hormonal response to prolonged use of SL-DIOL is unknown.

Chronic oral intake of androstenediol lowers serum high-density lipoprotein cholesterol concentrations (6, unpublished observations). Chronic androstenediol intake also elevates serum dihydrotestosterone concentrations (unpublished observations), 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. While 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. Sublingual cyclodextrin androstenediol also causes increases in serum estradiol and androstenedione concentrations. The effects of the altered hormonal milieu observed after sublingual cyclodextrin androstenediol intake on health or the adaptations to resistance training are not known.

References


Figure 1. Serum androstenedione concentrations after administration of placebo or sublingual cyclodextrin androstenediol (SL-DIOL).

* different from 0 and -20 min (P < 0.05)
Figure 2. Serum free testosterone concentrations after administration of placebo or sublingual cyclodextrin androstenediol (SL-DIOL).

* different from 0 and -20 min (P < 0.05)
Figure 3. Serum total testosterone concentrations after administration of placebo or sublingual cyclodextrin androstenediol (SL-DIOL).

* different from 0 and -20 min (P < 0.05)
Figure 4. Serum estradiol concentrations after administration of placebo or sublingual cyclodextrin androstenediol (SL-DIOL).

* different from 0 and -20 min (P < 0.05)
CHAPTER 7. CONCLUSIONS

The current findings indicate that there is an age difference in the serum testosterone response to testosterone precursor intake. In young men (23 y), ingesting 100 mg androstenedione t.i.d. does not alter serum testosterone concentrations, while in 30-60 year old men the same dosage of testosterone precursor causes an ~40% chronic increase in serum free testosterone. Since these older men had lower free and total testosterone concentrations than the previously investigated young men, it would appear that basal endogenous testosterone concentrations do alter the testosterone response to testosterone precursor intake. However, the physiologic significance of this moderate increase in free testosterone is unknown.

The findings of this project also demonstrate that there is no difference in the hormonal response to androstenedione, androstenedione plus DHEA, or androstenediol intake. Taking these data along with previous results, indicate that in normal 20-60 year old men, DHEA intake does not enhance serum testosterone or alter serum estrogen concentrations. In contrast to advertised claims, orally ingested androstenediol does not increase serum testosterone concentrations more effectively than androstenedione.

The current project also demonstrates that ingesting androstenedione or androstenediol increases serum estrogen and DHT concentrations. The increase in serum estradiol and DHT concentrations (~85 and 70%, respectively) associated with testosterone precursor intake in the present studies are close to the level used to experimentally induce BPH in dogs over a course of 9 weeks (~108 and 105%, for estradiol and DHT respectively), suggesting that prolonged use of testosterone precursors may cause untoward health
consequences. The serum testosterone, estradiol, and DHT response to androstenedione or androstenediol intake is not altered by the addition of saw palmetto, γLA, chrysin, I3C, or TT. These findings are in agreement with previous findings in young men.

Finally, the current data indicate that sublingual administration of smaller doses of androstenediol and androstenedione effectively bypasses digestive and hepatic catabolism, delivering the testosterone precursor to peripheral tissue where it can be converted into testosterone. This mode of delivery would appear to have more promise for testosterone enhancement than does an ingested product.

**Future Directions**

Although the current project answers some questions regarding the age related response to testosterone precursor intake, there are a great many questions that still need to be addressed. Future research in this area should evaluate the effects of higher doses of testosterone precursor intake since it is widely believed that athletes are taking much greater doses than have been currently studied. Therefore, future investigations should examine both the acute and long-term hormonal and physiologic responses to ingesting large doses (> 300 mg/day) of testosterone precursors.

It was observed that testosterone precursor intake causes changes in the hormonal and cholesterol milieu that may impair health. However, the long-term consequences of these changes are unknown. It is also unknown if these changes are reversed after cessation of testosterone precursor intake. Therefore, a fertile area for future research is in the area of the health consequences of long-term testosterone precursor intake.
Finally, since sublingual administration of 20 mg androstenediol acutely increases serum testosterone concentrations, it would be of interest to evaluate the time course for these changes, and the effects of long-term use of sublingual testosterone precursor.
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