Physiological and Hormonal Influences of Androstenedione Supplementation in Men 35 to 65 Years Old Participating in a High-Intensity Resistance Training Program

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Background: Since the passage of The Dietary Supplement Health and Education Act in 1994, there has been a flood of new “dietary” supplements promoting anti-aging benefits such as the enhancement of growth hormone or testosterone levels. Androstenediol and androstenedione are such products. This study’s purpose was to elucidate the physiological and hormonal effects of 200 mg/d of oral androstenediol and androstenedione supplemenation in men aged 35 to 65 years while participating in a 12-week high-intensity resistance training program.

Methods: Fifty men not consuming any androgenic-enhancing substances and with normal total testosterone levels, prostate-specific antigen, hemoglobin, and hematocrit, and with no sign of cardiovascular or metabolic diseases participated. Subjects were randomly assigned to a placebo, androstenediol (diol), or androstenedione (dione) group using a double-blind study design. Main outcomes included serum sex hormone profile, body composition assessment, muscular strength, and blood lipid profiles.

Results: During the 12 weeks of androstenedione or androstenediol use, a significant increase in the aromatization by-products estrone and estradiol was observed in both groups (P = .03). In the dione group, total testosterone levels significantly increased 16% after 1 month of use, but by the end of 12 weeks, they returned to pre-treatment levels. This return to baseline levels resulted from increases in aromatization and down-regulation in endogenous testosterone synthesis based on the fact that luteinizing hormone was attenuated 18% to 33% during the treatment period. Neither androstenediol nor androstenedione enhanced the adaptations to resistance training compared with placebo for body composition or muscular strength. However, both androstenediol and androstenedione supplementation adversely affected high-density lipoprotein cholesterol (HDL-C) levels, coronary heart disease risk (representing a 6.5% increase), and each group’s respective (low-density lipoprotein cholesterol [LDL-C]/HDL-C)/(apolipoprotein A/apolipoprotein B) lipid ratio (diol: +5.2%; dione: +10.5%; P = .05). In contrast, the placebo group’s HDL-C levels increased 5.1%, with a 12.3% decline in the (LDL-C/HDL-C)/(apolipoprotein A/apolipoprotein B) lipid ratio. These negative and positive lipid effects occurred despite no significant alterations in body composition or dietary intakes in the supplemental groups or placebo group, respectively.

Conclusions: Testosterone precursors do not enhance adaptations to resistance training when consumed in dosages recommended by manufacturers. Testosterone precursor supplementation does result in significant increases in estrogen-related compounds, dehydroepiandrosterone sulfate concentrations, down-regulation in testosterone synthesis, and unfavorable alterations in blood lipid and coronary heart disease risk profiles of men aged 35 to 65 years.

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A SIGNIFICANT decline in functional capacity with increasing age strongly correlates with a loss in muscle mass. Starting at age 30 years, a person will lose 10% of skeletal muscle content by age 60 years. Thereafter, muscle mass loss accelerates in most senior adults. In conjunction with changes in muscle mass, declines in several tissue-promoting hormones including insulin-like growth factor, growth hormone, testosterone, and calcitriolic factors occur. Whether the declines in these hormone levels directly cause skeletal muscle mass (SMM) losses or are simply a concomitant consequence of aging is unclear.

In hypogonadal men, testosterone replacement therapy enhances sexual performance, increases bone mass, improves nitrogen and protein balance, and may even improve memory retention in an aversive training paradigm. In addition, even in men with normal testosterone levels, exogenous testosterone injections (600
PARTICIPANTS AND METHODS

SUBJECT RECRUITMENT AND TREATMENT ASSIGNMENT

Seventy-one men volunteered to participate in this double-blind, placebo-controlled study. Prior to starting the treatment period, each subject completed a consent form approved by the institutional review board at ETSU and James H. Quillen–College of Medicine. Each subject participated in a prescreening blood profile for total testosterone levels, liver and kidney function, hemoglobin concentration, hematocrit, and prostate-specific antigen levels. If any of the specific initial markers were above the clinical reference values, subjects were not allowed to enter the study. Each subject was randomly assigned to a placebo, androstenediol (diol), or androstenedione (dione) treatment group. Androstenediol, androstenedione, or placebo (cornstarch) were administered twice daily (100 mg AM and 100 mg PM). Subjects were instructed to take 1 pill in the morning upon waking and 1 pill just prior to sleep. Pill counts were used to determine subject compliance.

PRETREATMENT AND POSTTREATMENT TESTING PROCEDURES

Participants completed a series of surveys including a health history survey, a standardized profile of mood states survey, a standardized interview for sexual function, a 3-day dietary and food frequency recall, and a standardized population-specific physical activity questionnaire. Physiological and biochemical related data collection included a pulmonary function test, a 12-lead resting electrocardiogram, a 12-lead maximal treadmill electrocardiogram with maximal oxygen consumption (VO2max) measured by indirect calorimetry, 9 skinfold and 8 circumference measurements, a single-frequency bioelectrical impedance for total body water, dual-frequency bioelectrical impedance for intracellular and extracellular water measurements, dual-photon x-ray absorption (DEXA) (bone density, total body fat content, regional body composition, and SMM estimates), a series of 1-repetition maximum strength tests (1-RM), a resting echocardiogram, a standardized clinical blood profile plus the assessment of hemoglobin A1c, and prostate-specific antigen, a hormonal profile series, and bone turnover assessment for osteoclastic and osteoblastic activity. This entire testing sequence was completed in 5 to 10 days. During posttesting, all subjects continued weight lifting and taking their respective supplements until all testing was completed. The main focus of this article was to present in detail from the complete variable listing outlined above only the data related to body composition, muscular strength, serum steroid hormone levels, and blood lipid profiles.

RESTING ELECTROCARDIOGRAM AND MAXIMAL TREADMILL PROCEDURES

All subjects performed a resting electrocardiogram and 12-lead exercise treadmill test at the start of the pretesting and posttesting procedures as previously described. The treadmill protocol was designed to allow a gradual warm-up period (3 minutes). If an individual had significant ST-segment changes during the treadmill tests, he was referred to a cardiologist for further testing and clearance to participate in the study.

BLOOD CHEMISTRY AND HORMONAL PROFILES

Blood samples were taken after a 12-hour fast. SmithKline Beecham Laboratories in Johnson City performed the standard blood chemistry analyses. Hormonal profile assessments were performed by the endocrinology laboratory at James H. Quillen–College of Medicine. Serum concentrations of dehydroepiandrosterone sulfate (DHEAS), androstenedione, free testosterone, total testosterone, estrone, estrogen, luteinizing hormone, follicle stimulating hormone, and serum-hormone binding protein were measured using either radioimmunoassay or enzyme-linked immunosorbent assay procedures (Diagnostic Systems Laboratories Inc, Webster, Tex). In regard to the blood draws, all remaining samples were taken within 1 hour of each person’s initial blood draw period. Each person completed 4 blood sampling periods (pretreatment, 1 month, 2 months, and posttreatment). Each month, total testosterone, hematocrit, hemoglobin, and prostate-specific antigen values were assessed to assure that no subject exceeded standardized clinical limits.

STRENGTH TESTING

Strength was assessed by testing each person’s 1-RM on the bench press, biceps curl, triceps press-down, leg extension, and leg curl as previously described. Prior to each person’s 1-RM test day, each subject was given an orientation and practice maximal test day so that he became accustomed to the testing procedures.

mg/wk) significantly increase muscle mass and strength. Thus, it has been suggested that an attenuation of the aging process or improvement in functional capacity may be possible if one can prevent the age-related decline in concentrations of hormones such as testosterone.

In 1994, Congress passed The Dietary Supplement Health and Education Act. This act has led to a flood of new “dietary” supplements promoting anti-aging benefits such as the enhancement of growth hormone or testosterone levels. In many cases, the advertisements for these products are misleading because few well-controlled clinical trials are available. More alarming is that many substances are being sold as safe and natural “dietary” alternatives for hormone enhancement. Androstenedione is such a product being marketed today to improve sexual performance, reduce body fat levels, and increase muscle mass. Androstenedione is a testosterone precursor classified as a prohormone. Thus, one could hypothesize that if consumed in stimulatory dosages, a significant increase in free and bound testosterone might occur. In a preliminary study by Mahesh and Greenblatt, 100 mg of oral androstenedione significantly increased blood testosterone levels in 2 women.
However, in June 1999, King et al10 published the first observations of taking oral androstenedione supplementation. In addition, regional and total SMM was calculated from the sum of DEXA arm and DEXA leg fat-free soft tissue (FFST) values (in kilograms). This method assumes that the arm FFST and leg FFST sum represents limb SMM, and that limb SMM represents 75% of the total body SMM.14,15 This technique has been validated in both young16 and aged men.17 As described above, total SMM was determined by DEXA (Lunar Corporation, Madison, Wis). To minimize testing error, all DEXA scans were performed by the same investigator to minimize tester error.

RISESTANCE TRAINING PROGRAM

Each subject worked out 3 days a week with an initial exercise intensity of 60% to 70% of his initial 1-RM results (week 1). Thereafter, exercise intensity ranged between 80% and 95% of each person’s initial 1-RM values. In some subjects, work intensities in the later weeks of the training program exceeded their respective pretraining maximum 1-RM on low-repetition (3-15 repetitions per set) workouts days. A series of abdominal and stabilizer exercises were developed using Swiss therapy balls to help prevent potential lower back injuries during the higher exercise intensities. All participants received a personalized training manual that precisely designated the exercises to be performed, the number of sets (2-3 sets), the number of repetitions (3-15 repetitions per set), and resistance loads for each exercise (Table 1). If a subject missed a scheduled training day, makeup sessions were provided within the week as needed. All training sessions were performed in the morning and evening hours at the ETSU athletic weight training facility.

One unique feature of the resistance training program was that a personal trainer supervised all training sessions. The personal trainer logged in each participants start time, assisted them through the workout (ensuring that repetitions, resistance, and sets were recorded properly), and then recorded each person’s finish time. The data were entered in a computer program specifically designed for the study, which calculated the total absolute amount of weight lifted, total workout minutes, and weight lifted in kilograms per minute on a daily, weekly, and cumulative basis. The reason such a rigorous evaluation method of the training program was 3-fold: (1) to ensure that training variations from one group to another did not affect the between-group comparisons, (2) to determine if androstenediol or androstenedione increased a person’s ability to train harder (ie, more work per unit time), and (3) to determine if androstenediol and androstenedione had any direct effects on the dependent measures of this study independent of the training load.

DIETARY ANALYSES

A 3-day dietary recall and food frequency intake surveys were given to each person. A registered dietitian carefully explained the procedures to each subject using a food model system. Subjects completed the food frequency intake survey in the laboratory while working with the dietitian. The 3-day dietary recall days were randomly assigned so that each person gave a feeding account of 1 weekend day and 2 weekdays. In addition, all subjects returned with their 3-day dietary recall all food labels from prepackaged foods (ie, candy bars) consumed for database updating as needed. The dietary recalls were used in this initial study to compare total kilocalorie intake, kilocalorie intake per unit of body weight, total grams of protein intake, and grams of protein per unit of body weight. The data were analyzed using the Dine Nutrient Analysis software (Dine Systems, Inc, Amherst, NY) for the 3-day dietary recalls and the Nutritionist 4 software (N-Square Computing, Salem, Ore) for the food frequency logs.

STATISTICAL ANALYSES

Data were analyzed using statistical software (DATADesk version 6.0; DATADesk, Ithaca, NY). Dependent variables were initially analyzed to determine whether there were any statistical differences among the groups before starting the intervention period. When no pretreatment significant differences were observed for a dependent variable, a 2-factor (between factor=treatment, repeated measure=time) analysis of variance (ANOVA) with repeated measures was used. When significant differences in a dependent variable among the treatment groups were observed prior to treatment, an analysis of covariance was used (ANCOVA) to account for these initial differences among the treatment groups as discussed in the “Results” section. When significant interaction effects were observed during either the ANOVA or ANCOVA procedures with repeated measures (P<.05), least-squares multiple comparison post hoc procedures were used to determine where differences occurred among the comparison cells.
cause estradiol and estrone were significantly elevated in the androstenedione treatment group.

Thus, the purpose of “The Andro Project” was to further elucidate the effects of oral androstenediol (a secondary pathway prohormone involved in testosterone synthesis and often found in combination with androstenedione in supplements) or androstenedione supplementation on muscle strength, body composition, hormonal profiles, kidney and liver function markers, basic clinical blood chemistry profiles, pulmonary function, cardiac function by echocardiography, bone density and turnover, dietary intake, psychological profiles of mood, and sexual performance in men aged 35 to 65 years. This project was designed in conjunction with a collaborative research team from The James H. Quillen–College of Medicine and East Tennessee State University (ETSU), Johnson City. Herein we present the results of the effects of androstenediol or androstenedione supplementation in combination with high-intensity resistance training on body composition, muscular strength, serum steroid hormone levels, and blood lipid profiles in men aged 35 to 65 years.

## RESULTS

Five subjects were not allowed to begin the study because of positive ST-segment depression or high blood pressure responses at rest or during the treadmill test. One subject was omitted because of abnormal mitral valve function results. Two subjects were dropped from the study after the first 3 weeks because of a lack of compliance regarding the training periods and designed program of intervention. Of the remaining 63 subjects who completed the first 4 training weeks, 12 more subjects dropped out of the study during the remaining 8 weeks. The reasons given for dropping out included a lack of time from a change in work schedules, belief that the training program was too intense, or a nonexercise-related injury or illness. Finally, 1 subject was removed from the study because his total testosterone levels exceeded the upper clinical limit for exclusion. Thus, the final data pool included 50 subjects (placebo, 18 men; diol, 17 men; and dione, 15 men).

Based on a power analysis, 12 subjects per group were needed to find a significant difference among the treatment groups when \( P \) was set to .05. Thus, despite the fact that 29.4% of the original sample pool did not complete the study, the results of this study are still valid.

### BASELINE CHARACTERISTICS

No significant differences were observed among the groups in any of the baseline characteristics (Table 2) or in hormonal profiles (Table 3) prior to starting the treatments and resistance training period. In regard to aero-

<table>
<thead>
<tr>
<th>Workout Weeks</th>
<th>Lifts Performed</th>
<th>Target Sets/Repetitions</th>
<th>Target Intensity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>Day 1: bench press, front military (seated), front lat pull-down, standing arm curl (cambered bar), cable triceps extension (V-bar), leg extension, leg press, standing calf raise, abdominal exercises</td>
<td>3/10</td>
<td>75-85</td>
</tr>
<tr>
<td></td>
<td>Day 3: bench press, front military (seated), front lat pull-down, standing arm curl (cambered bar), cable triceps extension (V-bar), leg extension, leg press, standing calf raise, abdominal exercises</td>
<td>3/10</td>
<td>75-85</td>
</tr>
<tr>
<td></td>
<td><strong>Special Note(s):</strong> all training days included 5 min of Swiss-ball stabilizing exercises</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-7</td>
<td>Day 1: same as weeks 1-3</td>
<td>3/3-5</td>
<td>85-95</td>
</tr>
<tr>
<td></td>
<td>Day 2: same as weeks 1-3</td>
<td>3/12-15</td>
<td>70-80</td>
</tr>
<tr>
<td></td>
<td>Day 3: same as weeks 1-3</td>
<td>3/8-10</td>
<td>80-85</td>
</tr>
<tr>
<td></td>
<td><strong>Special Note(s):</strong> all training days included 5 min of Swiss-ball stabilizing exercises; squat exercise was performed with free weights; abdominal exercises same as weeks 1-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Day 1: same as weeks 1-3</td>
<td>3/8</td>
<td>60-65</td>
</tr>
<tr>
<td></td>
<td>Day 2: no lifting; each subject stretched, walked 1.5 mi (2.4 km), and performed push-ups, pull-ups, and extra abdominal exercises</td>
<td>3/8</td>
<td>70-75</td>
</tr>
<tr>
<td></td>
<td>**Special Note(s): this week was considered an active rest week to prevent overtraining and injury; squat exercise was performed on a Smith machine or an equivalent machine (Bear Squat machine)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9-11</td>
<td>Day 1: same as weeks 1-3</td>
<td>3/3-5</td>
<td>85-95</td>
</tr>
<tr>
<td></td>
<td>Day 2: same as weeks 1-3</td>
<td>3/12-15</td>
<td>70-80</td>
</tr>
<tr>
<td></td>
<td>Day 3: same as weeks 1-3</td>
<td>3/8-10</td>
<td>80-85</td>
</tr>
<tr>
<td></td>
<td><strong>Special Note(s):</strong> all training days included 5 min of Swiss-ball stabilizing exercises; squat exercise was performed with free weights; abdominal exercises same as weeks 1-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Day 1: same as weeks 1-3</td>
<td>3/3-5</td>
<td>85-95</td>
</tr>
<tr>
<td></td>
<td>Day 2: same as weeks 1-3</td>
<td>3/12-15</td>
<td>70-80</td>
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<td></td>
<td>Day 3: same as weeks 1-3</td>
<td>3/8-10</td>
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<tr>
<td></td>
<td><strong>Special Note(s):</strong> all training days included 5 min of Swiss-ball stabilizing exercises; squat exercise was performed with free weights; abdominal exercises same as weeks 1-3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*For each exercise, the resistance was set so that each person needed spotter assistance on the next-to-last or last repetition.
bic capacity, as expected, \( \dot{V}O_2\text{max} \) did not significantly increase following the high-intensity resistance training program in any group.

HORMONAL RESPONSES TO ANDROSTENEDIOL OR ANDROSTENEDIONE SUPPLEMENTATION BEFORE AND AFTER THE TRAINING PERIOD

As expected, the dione group showed a significant elevation in serum androstenedione levels after 12 weeks of supplementation compared with the pretreatment value. Serum androstenedione levels increased 183% in the dione group. Compared with the dione and placebo groups, the dione group showed a significantly greater increase in serum androstenedione concentration. The dione group's androstenedione levels increased 62% from its pretreatment value. No significant change was observed in the placebo group.

No significant changes were observed in total testosterone, free testosterone, and serum hormone-binding globulin. However, estrone, estradiol, and DHEAS levels significantly increased in both the dione and dione groups. In addition, both the dione and placebo groups' estrone and estradiol levels were significantly greater than the placebo group following the intervention period. In slight contrast, the dione treatment produced the most significant increase in DHEAS levels (dione, 218%; dione, 61%) (Table 3).

ESTRADIOL AND TOTAL AND FREE TESTOSTERONE RESPONSES TO ANDROSTENEDIOL OR ANDROSTENEDIONE SUPPLEMENTATION FOR ALL MEASUREMENT PERIODS

Comparing a particular dependent variable at discrete points can be misleading, especially with an extended intervention period. This would be especially true regarding hormonal measurements, which are dynamic on a daily basis. Therefore, we examined the serum testosterone, estradiol, luteinizing hormone, and follicle-stimulating hormone levels at each blood sample period. To minimize the extrahormonal assay costs, based on the power analysis, 12 subjects were randomly selected from the original data set for month-by-month comparisons. For serum total testosterone levels, the dione group showed significantly higher total testosterone levels at months 1 and 2 compared with the dione and placebo groups (Figure 1A). However, starting at 1 month, the dione group's total testosterone levels began to decline while the levels in the dione and placebo groups gradually increased. Thus, by posttesting, there were no significant differences among the groups.

After 1 month of dione use, the serum free testosterone (SFT) level (Figure 1B) was 49.7% greater than that of the placebo group and neared significance \((P = .07)\). This difference occurred due to slight increase from pretraining levels (4.2%) in the dione group and a 3.3% decline in the placebo group's SFT levels. By month 2, the dione group's SFT levels were significantly greater than those of the placebo group \((P = .05)\). This difference occurred because in the placebo group, SFT levels declined from pretraining values 14.3% while the

Table 2. Baseline Subject Characteristics*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo Group ((n = 18))</th>
<th>Dione Group ((n = 17))</th>
<th>Dione Group ((n = 15))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>47.0 (8.4)</td>
<td>47.0 (8.7)</td>
<td>43.1 (6.5)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>87.9 (14.8)</td>
<td>89.6 (13.6)</td>
<td>88.0 (16.5)</td>
</tr>
<tr>
<td>BMI†</td>
<td>27.7 (3.5)</td>
<td>29.3 (4.1)</td>
<td>27.9 (4.1)</td>
</tr>
<tr>
<td>DEXA % body fat</td>
<td>20.4 (8.0)</td>
<td>19.9 (4.2)</td>
<td>18.6 (6.8)</td>
</tr>
<tr>
<td>DEXA FFM, kg</td>
<td>69.2 (7.8)</td>
<td>71.5 (9.4)</td>
<td>71.0 (3.2)</td>
</tr>
<tr>
<td>(\dot{V}O_2)max, mL·kg(^{-1}·\text{min}^{-1})*</td>
<td>39.0 (6.5)</td>
<td>38.1 (6.6)</td>
<td>39.6 (5.8)</td>
</tr>
<tr>
<td>Bench press 1-RM, kg</td>
<td>73.1 (25.9)</td>
<td>88.2 (41.6)</td>
<td>83.9 (28.4)</td>
</tr>
<tr>
<td>Leg extension 1-RM, kg</td>
<td>72.7 (23.3)</td>
<td>70.8 (21.7)</td>
<td>76.6 (22.5)</td>
</tr>
<tr>
<td>Total body strength, kg‡</td>
<td>276.5 (64.9)</td>
<td>298.9 (96.0)</td>
<td>302.0 (65.0)</td>
</tr>
<tr>
<td>Total body strength/DEXA FFM</td>
<td>3.98 (0.68)</td>
<td>4.11 (0.93)</td>
<td>4.29 (0.76)</td>
</tr>
</tbody>
</table>

*Data are given as mean (SD). Diol indicates androstenediol; dione, androstenedione; DEXA, dual-photon x-ray absorption; FFM, fat-free mass; \(\dot{V}O_2\)max, maximum oxygen consumption; and 1-RM, 1-repetition maximum strength test.
†Body mass index; calculated as weight in kilograms divided by the square of height in meters.
‡Total body strength = 1-RM total sum of the bench press, biceps curl, triceps press-down, leg extension, and leg curl.

Table 3. Hormonal Profiles Before and After Intervention Period*

<table>
<thead>
<tr>
<th>Hormone†</th>
<th>Pretreatment</th>
<th>Posttreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo Group ((n = 18))</td>
<td>Dione Group ((n = 17))</td>
</tr>
<tr>
<td></td>
<td>Placebo Group ((n = 15))</td>
<td>Dione Group ((n = 15))</td>
</tr>
<tr>
<td>Total testosterone, nmol/L</td>
<td>15.2 (7.0)</td>
<td>14.2 (5.2)</td>
</tr>
<tr>
<td>Free testosterone, pmol/L</td>
<td>54.9 (16.9)</td>
<td>61.6 (29.7)</td>
</tr>
<tr>
<td>Serum hormone-binding globulin, nmol/L</td>
<td>31.0 (16.2)</td>
<td>35.9 (25.6)</td>
</tr>
<tr>
<td>Estrone, pmol/L</td>
<td>109.6 (31.8)</td>
<td>109.5 (26.4)</td>
</tr>
<tr>
<td>Estradiol, pmol/L</td>
<td>131.3 (38.0)</td>
<td>142.1 (62.4)</td>
</tr>
<tr>
<td>Androstenedione, nmol/L</td>
<td>5.9 (2.1)</td>
<td>7.3 (8.0)</td>
</tr>
<tr>
<td>DHEAS, pmol/L</td>
<td>4.4 (2.2)</td>
<td>4.2 (2.3)</td>
</tr>
</tbody>
</table>

*Data are given as mean (SD). Diol indicates androstenediol; dione, androstenedione; and DHEAS, dehydroepiandrosterone sulfate.
†To convert total testosterone from nanomoles per liter to nanograms per deciliter, divide by 0.0347. To convert estradiol from picomoles per liter to picograms per milliliter, divide by 3.671.
‡Significantly different from placebo group \((P < .01)\).
§Significant pretreatment to posttreatment change \((P < .03)\).
‖Dio1 group vs dione group significantly different \((P < .004)\).
dione group returned to pretraining values after the temporary 1-month increase in SFT. Interestingly, the dione group had a significantly lower SFT level at the posttesting measurements compared with the 1-month measurement that was 15.4% lower (nonsignificant) than the pretesting measurement period while the placebo group was nearly identical to the pretreatment measurements. This decline in SFT indicated that less active testosterone was available after 3 months of continuous oral androstenedione use. In contrast, the diol group showed no significant SFT effect in the first 2 months of use. But, by the posttesting measurement period, the diol group had a significantly greater SFT level compared with the dione group (\(P < .05\)).

For the serum estradiol changes over time (Figure 2), the dione group’s estradiol levels significantly increased by month 1 and remained elevated throughout the study compared with placebo. The dione group did not show a significant increase in serum estradiol values until the final measurement period when free testosterone was significantly increased.

Finally, each subject’s individual measurement period mean was averaged together to obtain a 3-month cumulative value (1 month mean + 2 month mean + posttesting mean). The results indicated that estradiol was significantly elevated throughout the entire study period for both the diol and dione groups compared with the placebo group and with their initial pretreatment values. In contrast, only the dione group showed a trend toward a significant cumulative increase in serum total testosterone levels (\(P = .08\)) throughout the entire treatment period compared with the placebo group (Figure 3).

**BODY COMPOSITION AND DIETARY INTAKES**

Table 4 shows the results of DEXA body composition analysis. There were no significant changes observed. However, the dione group gained 3.6 kg in body weight and this resulted in a 6.0% relative increase in percentage of body fat (1.4% absolute gain in percentage of body fat). The results of the skinfold and circumference data indicated there were no significant differences among the groups. In addition, there were no significant differences in energy (kilocalories) or protein intake among the groups before or after the
12-week resistance training period. Total energy (caloric) intakes for the mean of the pretreatment and posttreatment measurements combined were 29.4 kcal/kg, 29.5 kcal/kg, and 28 kcal/kg for the dione, diol, and placebo groups, respectively. The percentage of calories derived from the different macronutrients for both the pretreatment and posttreatment measurements combined was as follows: placebo group = 53% carbohydrates, 16% protein, and 30% fat; diol group = 44% carbohydrates, 17% protein, and 38% fat; and dione group = 52% carbohydrates, 15% protein, and 32% fat. As for protein intake, the mean of the pretreatment and posttreatment measurements combined were 1.13 g/kg, 1.25 g/kg, and 1.14 g/kg for the placebo, diol, and dione groups, respectively.

Based on the training log data, both the placebo and diol groups worked out for 75 minutes per training day. For the dione group, the workout period lasted 72 minutes per day. The mean (SD) absolute quantity of weight lifted per workout per day was 10548 (2640) kg, 11991 (3119) kg, and 11749 (2241) kg for the placebo, diol, and dione groups, respectively. There were no significant differences in either the number of workout minutes or amount of absolute weight lifted per workout between the groups. As a result, there were no significant group differences in response to the resistance training program (Table 5). All 3 groups showed a similar significant increase in total strength and strength gain per to-
tal of each person’s FFM weight or per total of DEXA derived SMM. When the strength results for all groups are combined, the strength gains from pretesting to posttesting measures were 16.6% (upper body strength) to 29.1% (lower body strength). Because the initial training week was considered a warm-up period and the last week of training was set to taper each person’s workout to avoid posttraining results confounded by overtraining effects, the training workloads shown in Table 5 were calculated using weeks 2 to 11. Based on the training logs, the workloads among groups were not significantly different. There was a trend for the dione group to lift more weight in kilograms per minute than the placebo group (P < .05).

Finally, it is well recognized that adaptations to resistance training are dependent not only on the training stimulus but a person’s initial strength and training experience. Therefore, each group was divided into 2 distinct groups (group 1 = initial low strength; group 2 = initial high strength) based on the initial total strength measurements. An ANCOVA using initial strength as the covariant to account for individual subject differences for individual lifts (ie, bench press) and overall body strength (ie, total kilograms lifted for all lifts) was used to analyze the data. There were no significant differences between how each of the training groups improved as a result of the training program (Figure 4).

**BLOOD LIPID PROFILES**

At the start of the study, the diol group had significantly higher (P < .02) total cholesterol, low-density lipoprotein cholesterol (LDL-C), apolipoprotein (apo) A, and cardiac lipid risk profiles using the (LDL-C/HDL-C)/(apo A/apo B) ratio compared with the dione and placebo groups. Compared with the placebo and dione groups, the diol group’s lipid risk profile was 76% and 27% greater, respectively. This difference may be because the diol group consumed more total and saturated fats according to the 3-day diet records than both the placebo and dione groups (P = .05) throughout the study period. Triglyceride, HDL-C, and apo A levels were not significantly different among the groups. The initial data also indicated that the diol group had a greater number of small dense LDL-C molecules (high triglycerides with high LDL-C concentrations). To account for pretreatment differences, an ANCOVA procedure was used. The results indicated that after the treatment period, the placebo group’s cardiac lipid risk profile declined 12.3%, while the dione and dione groups increased 5.2% and 10.5%, respectively (Figure 5). A reduction mean (SD) in HDL-C occurred in both the dione (mean (SD) pretreatment HDL-C = 1.20 [0.23] mmol/L; posttreatment HDL-C = 1.12 [0.26] mmol/L) and dione (pretreatment HDL-C = 1.21 [0.23] mmol/L; posttreatment HDL-C = 1.15 [0.27] mmol/L) groups, whereas the placebo group showed an increase in HDL-C (pretreatment HDL-C = 1.18 [0.33] mmol/L; posttreatment HDL-C = 1.24 [0.30] mmol/L) following the training program.

These results indicate that neither oral androstenediol nor androstenedione supplementation has any significant effects on enhancing the adaptive responses to 12 weeks of high-intensity resistance training in men 35 to 65 years old. However, results of this study strongly suggest that oral testosterone precursor supplementation leads to abnormally large and significant increases in aromatization by-products, such as estradiol and estrone, with significant increases in serum DHEAS levels from pretreatment values. In addition, these data indicate that within 1 month of dione use, endogenous testosterone production is down-regulated based on a 33% attenuation in luteinizing hormone serum levels. Finally, the dione and dione groups’ (LDL-C/HDL-C)/(apo A/apo B) lipid risk ratio significantly increased 10.5% and 5.2%, respectively, while the placebo group showed a lower risk ratio by 12.3% following the 12 weeks of high-intensity resistance training.

In the present study, it could be argued that the lack of enhanced adaptive responses to resistance training resulted from inadequate supplemental dosages since no significant difference in testosterone levels were observed at the end of the study. However, serum androstenediol and androstenedione groups. Data are given as mean (SEM). Asterisk indicates significant strength improvement from pretreatment period, P < .05.
testosterone synthesis is testicular. Thus, if androgen lev-

levels. One might speculate that with additional training

the use of androstenediol or androstenedione since nei-

androstenedione supplementation may

fluctuation in resistance training adaptation with

not significantly different from pretreatment values. One might speculate that with additional training time, these gradual increases in total testosterone and es-

androstenedione levels increased 62% and 183% in the diol and dione groups, respectively, indicating adequate testos-

in young men. The difference in results between the studies may relate to the distinct age difference in populations. One would hypothesize that an older population of men would most likely benefit from testosterone precursor supplementation since testosterone synthesis declines after the age of 30 years. However, despite the apparent effect on total testosterone that was observed in this study, as was observed in the study by King et al., there were no significant increases in free testosterone that could account for why the changes in strength and body composition were independent of supplementation as previously reported. Ninety-eight percent of the body’s total testosterone is protein bound. Ninety-eight percent of the body's total testosterone is protein bound. However, unbound testosterone is most metabolically ac-

androstenedione levels increased 62% and 183% in the diol and dione group, supplementation did alter total testos-

terone precursor supplementation since testoster-

an enhancement in testosterone production but a sus-

The results partially conflict with those of King et al., who showed that despite a 100% increase in serum androstenedione levels, neither free nor total testos-

If one synthesis declines after the age of 30 years. How-

of the aromatization process. Other physiological conditions, 80% of estrogens are peripherally synthesized while 95% of tes-

The table indicates that after placebo conditions, changes in total testosterone levels were positively correlated with a change in luteinizing hormone (P = .002) and estradiol (P = .04); leptin (P = .04) concentrations were negatively correlated (R^2 = .59.8%). It can be speculated that under continued high-intensity resistance training conditions, an increase in free testosterone levels may occur to meet training protein synthesis turnover demands. In addition, resistance training has clearly been shown to enhance the formation of bone when training exceeds 6 months. Thus, it is not surprising that there was a positive relationship between estradiol and serum testosterone. The negative correlation between leptin and total testosterone changes may be because there was a small, but nonsignificant reduction in abdominal body fat indicated by a nonsignificant reduction in the placebo group’s waist-to-hip ratio following the intervention period. Previous research indicates a significant negative correlation between abdominal body fat and serum total and free testosterone concentrations.

Looking at the dione and diol groups individually, the stepwise multiple regression analysis indicates that serum androstenedione concentration was the first loading factor for the diol group, while in the diol group, DHEAS loaded first into the stepwise multiple regression model. These findings support the hypothesis that androstenedione and androstenediol alter a male's hormonal profile in different ways. In the diol group, it is important to note that there was a negative correlation between a change in luteinizing hormone concentrations and serum total testosterone levels. After accounting for the other factors in the model, changes in lutein-
Luteinizing hormone concentration accounted for 33.9% of the total testosterone change while a change in serum androstenedione concentrations accounted for 38.8% of total testosterone change. These results indicate that when the conversion of testosterone to estrogen can no longer prevent negative inhibition resulting from a backup of pretestosterone prohormone precursors, as expected, a person's luteinizing hormone concentrations are downregulated. This is evident by the fact that luteinizing hormone secretion declined 33% within 1 month of dione use. In addition, even after total testosterone levels returned to baseline following 3 months of dione use, serum luteinizing hormone levels remained attenuated 18% (Figure 6).

In the present study, the resistance training program produced equally significant gains in strength for all treatment groups. Thus, the lack of any significant differences between the treatment groups for changes in relative body fat, fat-free mass, and estimates of total body SMM are not related to an inadequate training stimulus. These body composition results conflict with those of King et al,10 who showed that both fat mass and stimulus. These body composition results conflict with body SMM are not related to an inadequate training relative body fat, fat-free mass, and estimates of total all treatment groups. Thus, the lack of any significant changes. This is evident by the fact that the sum of all changes were determined using DEXA procedures, a procedure sensitivity could not detect these changes. This is evident by the fact that the sum of all circumference sites increased about 1.7% while the skinfold sums decreased approximately 5% for all treatment groups. These results indirectly suggest that some muscle mass hypertrophy occurred since subcutaneous body fat declined while circumferences (hypertrophy) increased at the same anatomical locations. However, it is important to point out that skinfold measurements have previously been shown to have only modest sensiti-
tions decreased 0.06 mmol/L (2.3 mg/dL), indicating a 6.9% increase in risk of coronary heart disease while the placebo group showed a 6.9% decline in risk according the Framingham data of Gordon et al.34

Interestingly, one would suspect that because estrogen levels were elevated as a result of androstenedione and androstenediol use that positive effects might be incurred regarding a person's blood lipid profiles. However, although estrogen and positive blood lipid profiles are clearly established in literature, in the present study, one must keep in mind how most of the estrogen increases occurred in the supplemental groups. Under placebo conditions, estradiol levels increased 17% from baseline values. Thus, one could consider this change to be the appropriate hormonal adaptation required for meeting physiological needs of the current resistance training program (ie, an increase in positive bone turnover). In the dione and diol groups, however, estradiol levels increased 92.1% and 57.4%, respectively. Thus, over and above the normal estradiol response to resistance training, androstenedione and androstenediol use dramatically increased estradiol levels beyond each group's physiological adaptive needs. Considering that one of the primary functions of endogenous cholesterol synthesis is steroid hormone production, it is easy to understand how artificial enhancement of estradiol levels could worsen blood lipid profiles as occurred in this study. With attenuation in cholesterol conversion to steroid-based hormones, HDL-C production is down-regulated because the normal avenue for cholesterol removal, steroid hormone production, has been removed due to the oral pro-hormone supplementation.

Epidemiological data suggest that as a male's testosterone levels decline with age, cardiovascular disease risk factors increase.3 Also, it is clear that responses to any intervention are individualized. Thus, in the present study, each treatment group was subdivided into low and high responders based on the median value of each group's total testosterone change. In the placebo group, when total testosterone levels significantly increased (high responder), a significant 26.2% reduction in lipid risk profiles was observed compared with a nonsignificant change in the low-responder placebo subgroup. In contrast, the dione group high responders showed no significant lipid ratio improvement while the low responders showed a 20.2% unfavorable change. These data suggest that positive lifestyle changes that enhance a male's testosterone levels may improve cardiovascular disease risk profiles. But, when artificial enhancement of male hormone profiles are attempted, dramatic increases in cardiovascular disease risk profiles can occur, especially in individuals who do not respond to the treatment intervention.

It is important to point out that in the present study and the study by King et al.,10 isolated supplements were used; while many over-the-counter products are a combination of factors termed stacking. Some of these formulas claim to include aromatization inhibitors. However, a recent report indicates that these claims may not be supported by recent empirical data from a well-controlled clinical trial.33 Therefore, one can suspect that when stacking formulas are consumed, despite attempts to block estrogen production, the potential negative effects of multiple testosterone precursors would be more severe. This is especially true when one considers that an individual's use of these substances is totally unregulated and concentrations greater than recommended can be easily consumed.

In conclusion, the results of this study indicate that testosterone precursors, such as androstenediol and androstenedione, do not enhance a man's adaptations to resistance training when consumed in dosages as recommended by manufacturers. But testosterone precursor supplementation does result in significant increases in estrogen-related compounds, increases in DHEAS concentrations, down-regulation in an individual's testosterone synthesis, and unfavorable alterations in a person's blood lipid and coronary heart disease risk profiles. Finally, the results of this study indicate that the potential for additional negative side effects are possible when manufacturers can freely stack various forms of testosterone precursors and unregulated individual use of these products continues to occur under The Dietary Supplement Health and Education Act. It is our opinion that until further research clarifies the functional roles and potential risk factors of oral testosterone precursors that unmonitored use of these substances is not recommended. In addition, if an individual chooses to use testosterone precursors, it is essential that regular monitoring of serum hormone levels, and blood lipid profiles including HDL-C, LDL-C, and apolipoproteins, should occur.

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