
PRODUCT TESTING

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Effects of Anabolic Precursors on Serum Testosterone Concentrations and Adaptations to Resistance Training in Young Men

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The effects of androgen precursors, combined with herbal extracts designed to enhance testosterone formation and reduce conversion of androgens to estrogens was studied in young men. Subjects performed 3 days of resistance training per week for 8 weeks. Each day during Weeks 1, 2, 4, 5, 7, and 8, subjects consumed either placebo (PL; $n = 10$) or a supplement (ANDRO-6; $n = 10$), which contained daily doses of 300 mg androstenedione, 150 mg DHEA, 750 mg Tribulus terrestris, 625 mg Chrysin, 300 mg Indole-3-carbinol, and 540 mg Saw palmetto. Serum androstenedione concentrations were higher in ANDRO-6 after 2, 5, and 8 weeks ($p < .05$), while serum concentrations of free and total testosterone were unchanged in both groups. Serum estradiol was elevated at Weeks 2, 5, and 8 in ANDRO-6 ($p < .05$), and serum estrone was elevated at Weeks 5 and 8 ($p < .05$). Muscle strength increased ($p < .05$) similarly from Weeks 0 to 4, and again from Weeks 4 to 8 in both treatment groups. The acute effect of one third of the daily dose of ANDRO-6 and PL was studied in 10 men (23 ± 4 years). Serum androstenedione concentrations were elevated ($p < .05$) in ANDRO-6 from 150 to 360 min after ingestion, while serum free or total testosterone concentrations were unchanged. These data provide evidence that the addition of these herbal extracts to androstenedione does not result in increased serum testosterone concentrations, reduce the estrogenic effect of androstenedione, and does not augment the adaptations to resistance training.

Key Words: androstenedione, DHEA, saw palmetto, indole-3-carbinol, chrysin, Tribulus terrestris

Androstenedione and dehydroepiandrosterone (DHEA) are weak androgenic steroids capable of being converted to testosterone (24, 28, 34). While ingestion of

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DHEA (25, 29, 30) and androstenedione (25) may increase serum testosterone concentrations in women, we have recently observed that ingestion of androstenedione (21) and dehydroepiandrosterone (DHEA) (7) does not increase serum testosterone concentrations or augment the adaptations to resistance training in young men. Androstenedione ingestion, however, increased serum estrogen concentrations, and reduced serum high-density lipoprotein cholesterol (HDL-C) concentration.

Several herbal extracts have been shown to alter steroid metabolism. Indole-3-carbinol is an extract from cruciferous vegetables that has been shown to enhance oxidative metabolism and excretion of estrogens (26, 27). Chrysin, a flavanoid from *Passiflora caerulea*, has also been shown to inhibit aromatization *in vitro* (20). Saw palmetto (*Serena repens* B) extract has been shown to inhibit 5α -reductase activity (10, 35). Although ingestion of an extract from *Tribulus terrestris* has been claimed to increase serum testosterone concentrations subsequent to increased serum LH concentrations, scientific evidence is lacking. Ingesting these herbal extracts in conjunction with DHEA and androstenedione have been speculated to prevent 5α -reduction limiting the ingested androgens to the 17β hydroxysteroid dehydrogenase (17β -HSD) pathway (28), thereby promoting testosterone formation and minimizing estrogen and dihydrotestosterone (DHT) formation. One purpose of this study was to determine whether the addition of these herbal extracts to a supplement containing DHEA and androstenedione taken acutely or chronically increases serum testosterone concentrations or enhances the adaptations to resistance training in healthy young men. A second purpose of this study was to determine whether the addition of these herbal extracts prevents the formation of estrogens, or reduced HDL-C concentrations observed with chronic androstenedione intake.

Methods

Subjects

A total of 30 healthy young (19–29 years; Table 1) males were recruited for this study, which was approved by the Iowa State University Human Subjects Committee. All participants completed a written questionnaire regarding the use of nutritional supplements or participation in a resistance-training program prior to enrollment in this study. Subjects did not report any current or previous supplement use or resistance training. All subjects were free of cardiovascular or orthopedic conditions that would contraindicate exercise.

Acute Administration of Andro-6

The effects of acute ingestion of Andro-6 (ANDRO-6) on the serum concentrations of androstenedione, free testosterone, total testosterone, estradiol (E_2), LH, and follicle stimulating hormone (FSH) were studied in 10 of the men (23 ± 4 years). On two occasions separated by at least 1 week, and after an overnight fast, subjects ingested one third of the daily dose of ANDRO-6 or placebo (PL: 83 mg rice flour), administered in a randomly assigned double-blind manner. Blood samples were obtained before and every 30 min for 6 hr after ingestion. Serum hormone concentrations were determined as described below.

Table 1 Anthropometric Data During 8 Weeks of Resistance Training and Supplementation

Variable	Week	PL (n = 10)	ANDRO-6 (n = 10)
Age (years)		23.3 ± 1.1	21.6 ± 0.7
Height (cm)		178.0 ± 1.5	182.7 ± 1.8
Body mass (kg)	0	81.1 ± 5.2	85.1 ± 5.3
	*4	83.3 ± 5.1	87.2 ± 5.3
	*8	83.2 ± 4.9	86.4 ± 4.9
Body fat (%)	0	21.3 ± 1.9	24.4 ± 2.4
	*8	19.9 ± 2.1	23.1 ± 2.5
Fat mass (kg)	0	18.0 ± 2.9	21.3 ± 3.1
	*8	17.2 ± 2.9	20.3 ± 2.8
Lean mass (kg)	0	63.1 ± 2.6	63.8 ± 3.5
	*8	66.0 ± 2.5	66.1 ± 3.6
Waist:hip ratio	0	0.84 ± 0.0	0.84 ± 0.01
	*4	0.85 ± 0.0	0.84 ± 0.01
	*8	0.86 ± 0.0	0.85 ± 0.01
BMI (kg/m ²)	0	25.6 ± 1.6	25.5 ± 1.5
	*4	26.3 ± 1.6	26.1 ± 1.5
	*8	26.3 ± 1.5	25.9 ± 1.3

*Significantly different from Week 0 for all groups (main effect; $p < .05$)

Andro-6 Supplementation During Resistance Training

After screening, 20 of the men were randomly assigned in a double blind manner to groups that ingested either ANDRO-6 (containing 150 mg DHEA, 300 mg androstenedione, 300 mg indole-3-carbinol, 540 mg Saw palmetto, and 750 mg Tribulus terrestris) or placebo (250 mg rice flour) daily during Weeks 1–2, 4–5, and 7–8 during 8 weeks of resistance training. Supplementation was administered in a pattern of 2 weeks on supplements followed by 1 week off, to simulate the supplementation regimen recommended by the manufacturer of ANDRO-6. This cyclical pattern is thought to allow for a washout period reducing the likelihood of negative side effects such as gynecomastia and cholesterol abnormalities associated with androgenic supplement usage. Supplements were taken in three equal doses before 9:00 AM, at 3:00 PM, and at bedtime. Andro-6 (Experimental and Applied Sciences, Golden, CO) is a commercially available product, designed to produce a timed release effect of the active ingredients. The DHEA and androstenedione in ANDRO-6 were derived from wild yams and were assayed to be 98% and 99% pure by high performance liquid chromatography by two independent laboratories (Biomedical Laboratories, Petaluma, CA, and Integrated Biomolecule, Tucson, AZ, respectively). All other components of ANDRO-6 were assayed for purity, and the ANDRO-6 tablet was assayed to verify content (Integrated Biomolecule, Tucson, AZ). In

order to encourage compliance, subjects maintained a record of supplement ingestion and were required to return unused supplements at the completion of the study.

Resistance Training

Subjects performed resistance training 3 days per week on non-consecutive days using bench press, shoulder press, knee extension, right and left knee flexion, vertical butterfly, leg press, calf press, biceps curl, triceps curl, and latissimus dorsi pulldown. Subjects were instructed on proper lifting technique and supervised during all lifting sessions. The resistance-training program was designed to increase muscular strength in all major muscle groups of the body. Subjects performed three sets of 10 repetitions for the first 2 weeks of resistance training. For the final 6 weeks of training, subjects performed three sets of 8 repetitions. Resistance was established at 80–85% of the untrained one repetition maximum (1-RM). Following the determination of 1-RM after 4 weeks of training, the training intensity was adjusted to 80–85% of the new 1-RM. The amount of weight lifted, and the number of sets and repetitions performed was recorded for each training session. Subjects were instructed to limit exercise to the prescribed training regimen throughout the study.

Strength Testing

Before training, and after 4 and 8 weeks, subjects were tested for 1-RM. Subjects were allowed a brief light resistance warm-up, then encouraged to meet their 1-RM within five trials of increasing resistance (2). One repetition maximum was assessed on bench press, shoulder press, knee extension, right and left knee flexion, biceps curl, triceps extension, latissimus dorsi pulldown, and vertical butterfly. All resistance training and 1-RM testing was performed on multistation isotonic resistance equipment (FTX, Paramount Fitness Equipment, Los Angeles, CA).

Body Composition

Height, weight, and body circumferences were determined before training, and after Weeks 4 and 8. Circumferences were measured by the same investigator at the shoulders, biceps, chest, abdomen, waist, hips, gluteal, thigh, and calf using methods specified by the American College of Sports Medicine (2). Body composition was determined through hydrostatic weighing using a computer interfaced load cell and custom computer program before training and after 8 weeks of training and supplementation. The computer program utilizes the Siri equation for body fat (33), and Goldman-Becklake equation for residual lung volume (15).

Clinical Blood Chemistry and Hormonal Analyses

Blood samples were obtained after an overnight fast for standard blood chemistry and hormonal analyses before training and after 2, 5, and 8 weeks of training and supplementation. Blood was withdrawn without stasis from a catheter inserted into an antecubital vein. A commercial laboratory (Labcorp, Kansas City, MO) performed all clinical blood chemistry analyses. Another blood sample was centrifuged and serum was frozen at -80°C until analysis. Serum concentrations of free and total testosterone, androstenedione, estrone (E_1), estradiol (E_2), and estriol (E_3)

were measured with radioimmunoassay (RIA) using commercially available kits (Diagnostic Products, Los Angeles, and Diagnostic Systems Laboratories, Webster, TX). Serum concentrations of LH and FSH were measured using commercially available immunoradiometric assay (IRMA) kits (Diagnostic Products, Los Angeles, CA). All samples for each subject were assayed in duplicate in the same assay. The intra-assay coefficients of variation for each assay were less than 9.0%.

Oral Glucose Tolerance Test

Before training and again within 3 days (48 ± 1 hr) following the last training session, the subjects underwent a 2 hr, 75 g oral glucose tolerance test (OGTT) between 5 and 10 AM, after an 8–12-hr fast. Blood samples (approximately 10 ml) were obtained at 0, 30, 60, 90, and 120 min from a flexible catheter inserted into an antecubital vein. Plasma was separated and stored at -80°C until analysis for glucose and insulin concentrations. Plasma glucose concentrations were measured spectrophotometrically using the hexokinase method (Sigma Chemical, St. Louis, MO). Plasma insulin concentrations were determined via RIA (Diagnostic Products, Los Angeles, CA). The intra-assay coefficient of variation (CV) for insulin was 7.1%.

Subjects were given written instructions for a 150 g per day carbohydrate diet and instructed to otherwise maintain their regular diet. Diet records were maintained for 3 days prior to the initial OGTT, and subjects were provided copies of the initial diet record and asked to repeat the same diet for the post training OGTT. Dietary records were assessed for composition using food analysis software (Food Comp, Iowa State University, Ames, IA). Subjects were also instructed to maintain their usual dietary regimen, were frequently reminded during the course of the study to maintain their normal diet, and all indicated that their dietary practices did not change during the study period.

Muscle Histochemistry

Muscle biopsies were obtained before and after resistance training for determination of skeletal muscle fiber type distribution and mean cross-sectional area. Muscle samples (~100 mg) were obtained from the lateral aspect of the vastus lateralis muscle of the subjects, using the needle biopsy technique described by Bergstrom (5). Muscle specimens were placed in mounting medium and frozen in isopentane cooled to the temperature of liquid nitrogen for later sectioning and staining. Frozen transverse sections (~10 μm) were cut on a histostat (AO Scientific Instruments, Histostat Microtome) at -20°C . The percent of Type I and Type II muscle fibers were determined in sections stained for adenosine triphosphatase activity at pH 9.4 after a preincubation at pH 4.3. In addition, the samples were counter stained with an Eosin Y stain for color enhancement to aid in the image analysis of the different muscle fiber types. Muscle fiber type distribution and muscle fiber areas were determined using a computer operated image analysis system (Neosis Visilog Image Analysis Software; SGI-Computer; Sony DXC-3000A-Camera). The system captures the light microscope image, thresholds the images, traces the muscle fiber boundaries, counts the light and dark muscle fibers, and measures the cross sectional areas of all muscle fibers. To determine the percentage distribution of Type I and

Type II fibers, all viable fibers (406 ± 47) were used. For determination of mean cross-sectional area of Type 1 and Type 2 fibers, groupings of clearly delineated fibers were highlighted, and a technician blinded to the treatments randomly selected 20 fibers of each type for each specimen. This sampling technique produces values for fiber area that agree with those obtained from measurements of the whole field (R.A. Fielding, personal communication).

Calculations and Statistics

Incremental areas under the curve for insulin and glucose were calculated using the trapezoidal model with a custom computer program. Statistical analysis was performed using a two-way repeated measure analysis of variance. When significant interactions were observed, specific mean differences were located with a Newman-Keuls multiple comparison test. All statistical tests were performed with commercial software (Sigma Stat 1.0, Jandel Scientific, San Rafael, CA), and were evaluated at $p < .05$.

Results

Subjects

This study was part of a larger investigation involving various supplements, and the data from PL have been previously reported elsewhere (7, 21). Treatment groups were similar in terms of fitness, age, and exercise experience.

Acute Hormonal Response to ANDRO-6 Administration

Ingestion of one third of the daily dose of ANDRO-6 resulted in increased serum androstenedione concentrations within 150 min (Figure 1; $p < .05$) that peaked at 196% above baseline at 300 min after ingestion. Serum androstenedione concentrations tended to decline after 300 min, but remained elevated at 360 min in ANDRO-6 ($p < .05$). Serum concentrations of LH and FSH were not altered during the 360 min following ANDRO-6 or PL consumption. ANDRO-6, or PL did not alter the serum concentrations of free testosterone or total testosterone (Figure 2). Serum estradiol concentrations increased after 60 min in PL and ANDRO-6 ($p < .05$; main effect for time) and were not different between the two groups.

Resistance Training

There were no significant differences between PL and ANDRO-6 in the number of sets or repetitions performed per exercise session, or the relative intensity of each exercise session. When the data from all exercises are combined, subjects exercised at an intensity of $81 \pm 2\%$ of 1-RM during the first 4 weeks of training and at $83 \pm 1\%$ of 1-RM for the final 4 weeks of training. The total mean force produced per subject each day was 46 ± 1 kN during the first 4 weeks of training and 43 ± 1 kN during the final 4 weeks of training, with no differences observed between groups. The lower amount of force produced during Weeks 4–8 was due to the lower number of repetitions performed during each exercise session.

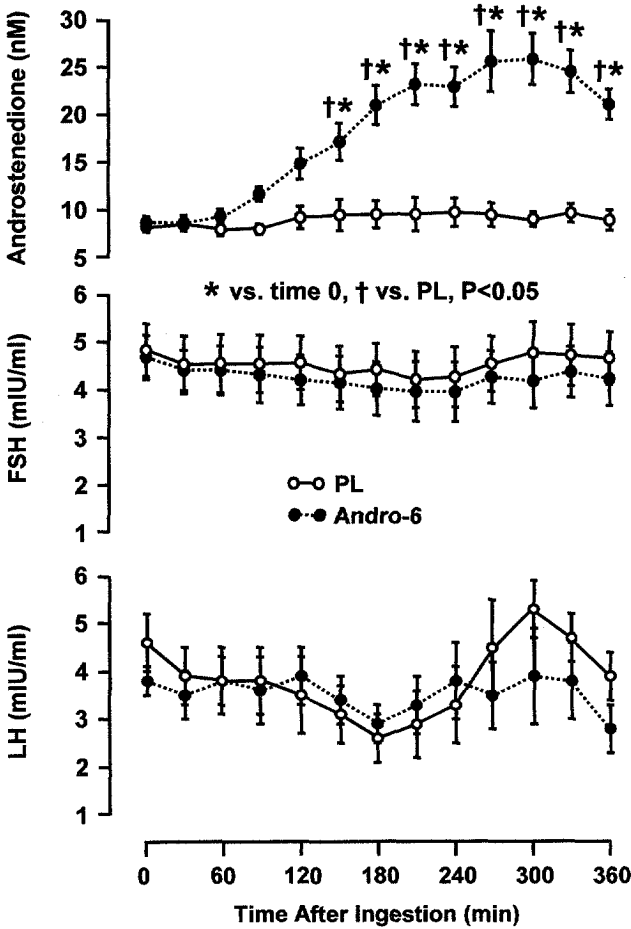


Figure 1 — Serum androstenedione, LH, and FSH concentrations after ingestion of Andro-6 or placebo (PL). Data are means \pm SE for $n = 10$. Note: *Significantly different from time 0 for Andro-6. †Significantly different from placebo ($p < .05$).

Hormonal Response to Andro-6 Administration During Resistance Training

Due to a loss of power to the laboratory freezer and thawing of serum samples, we were unable to measure serum concentrations of DHT.

Serum androstenedione concentrations (Figure 3) increased in ANDRO-6 at Week 2 (26 ± 4 nM), Week 5 (27 ± 3 nM), and Week 8 (21 ± 4 nM) compared to Week 0 (10 ± 1 nM; $p < .05$). Serum androstenedione concentrations were unaffected by training and supplementation in PL. The calculated effect size for the comparison of the androstenedione concentrations was large (0.88). This calculation highlights the effectiveness of ANDRO-6 in increasing serum androstenedione concentrations.

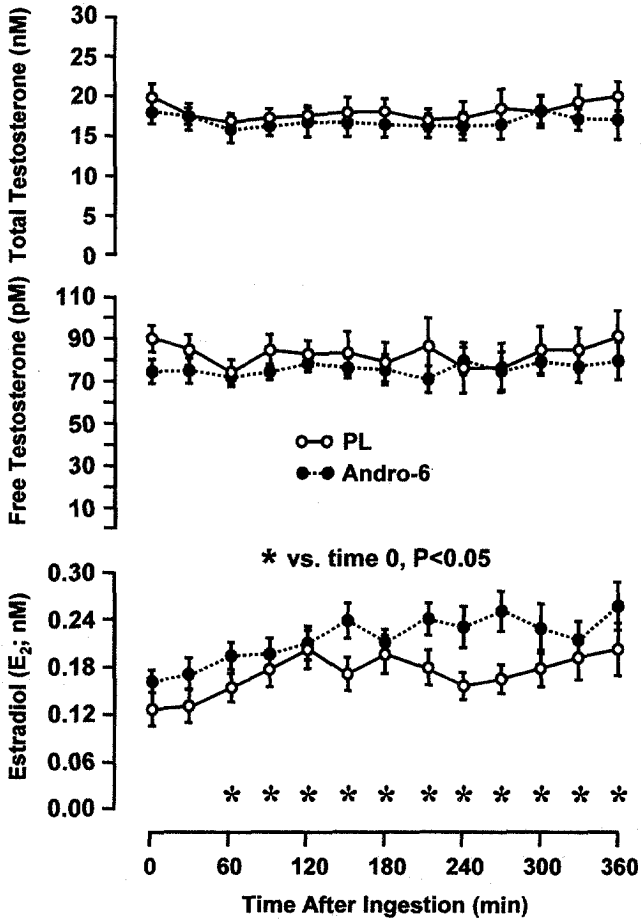


Figure 2 — Serum free and total testosterone, and estradiol concentrations after ingestion of Andro-6 or placebo (PL). Data are means \pm SE for $n = 10$. Note: *Significantly different from time 0 for estradiol (main effect; $p < .05$).

Serum concentrations of LH were not altered by supplementation and training in ANDRO-6 or PL (Figure 3). Supplementation and training also did not alter serum FSH concentrations in ANDRO-6 or PL.

Serum free and total testosterone concentrations (Figure 4) were not altered by 8 weeks of resistance training and supplementation in either PL or ANDRO-6. The calculated effect size for the comparison of the free testosterone data for Week 0 versus Week 2 was very small (0.03). Assuming a power of 80% and a $p = .05$, a sample size of 9,537 would be required to detect a significant increase in the serum testosterone concentration.

Because one subject in PL had initial serum estradiol concentrations more than three standard deviations away from the group mean (0.46 nM), his data were excluded from the statistical analysis of serum estradiol concentrations. Serum

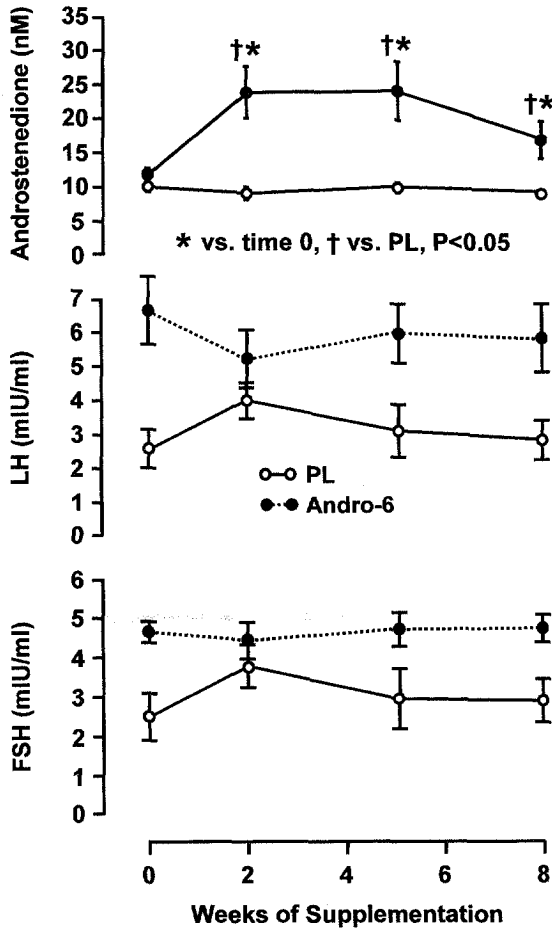


Figure 3 — Serum androstenedione, LH, and FSH concentrations before and during resistance training combined with 300 mg/day androstenedione (ASD, $n = 9$) or placebo (PL, $n = 10$) supplementation. Data are means \pm SE for $n = 10$. Note: *Significantly different from Week 0 for Andro-6. †Significantly different from placebo ($p < .05$).

estradiol, estrone, and estriol concentrations were not changed in PL. Serum estradiol concentrations increased ($p < .05$) after 2, 5, and 8 weeks training and supplementation in ANDRO-6. Serum estrone concentrations were elevated ($p < .05$) at Week 5 and Week 8 in ANDRO-6 (Figure 5).

Muscle Strength

Muscle strength did not differ between PL and ANDRO-6 before training, or after 4 or 8 weeks of training. To facilitate data presentation (Figure 6), upper body strength was calculated as the sum of 1-RM for bench press, shoulder press, and latissimus dorsi pulldown. Upper body strength increased similarly in PL and ANDRO-6 after 4 weeks of resistance training and supplementation (main effect; $p < .05$). Upper

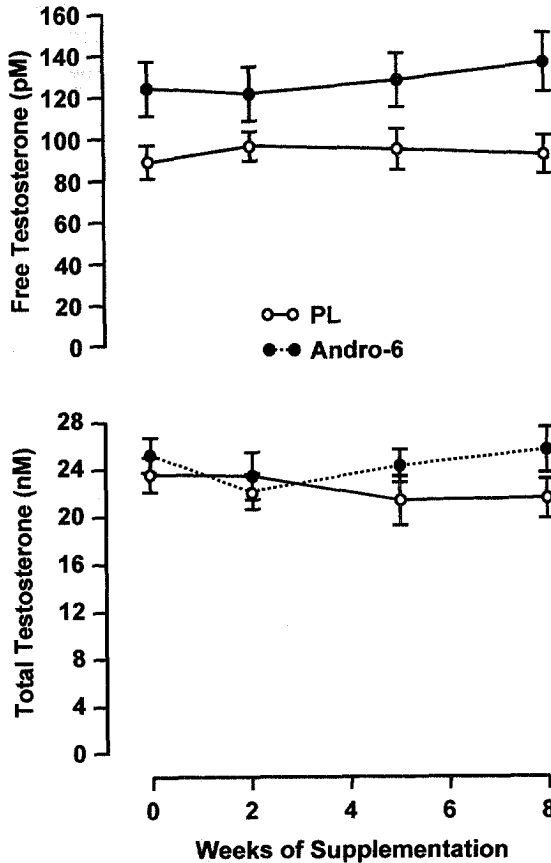


Figure 4 — Serum free and total testosterone concentrations before and during resistance training combined with Andro-6 or placebo (PL) supplementation. Data are means \pm SE for $n = 10$.

body strength further increased in all groups during the final 4 weeks of resistance training and supplementation ($p < .05$; main effect). The overall increase in upper body strength from Week 0 to Week 8 did not differ in PL ($2,016 \pm 170$ vs. $2,492 \pm 203$ N) and ANDRO-6 ($1,777 \pm 170$ vs. $2,093 \pm 181$ N). Lower body strength, calculated as the sum of knee extension, right and left knee flexion, increased similarly during the first 4 weeks of resistance training and supplementation in PL and ANDRO-6 (main effect; $p < .05$). The final 4 weeks of resistance training and supplementation resulted in additional increases in lower body strength in both groups (main effect; $p < .05$). The overall increase in lower body strength from Week 0 to Week 8 did not differ in PL ($1,387 \pm 55$ vs. $1,980 \pm 70$ N) or ANDRO-6 ($1,556 \pm 88$ vs. $2,062 \pm 90$ N). When the data for both groups and all exercises are combined, mean whole body muscle strength increased $18 \pm 2\%$ for the first 4 weeks of training, while the final 4 weeks of resistance training resulted in additionally increased strength of $10 \pm 2\%$.

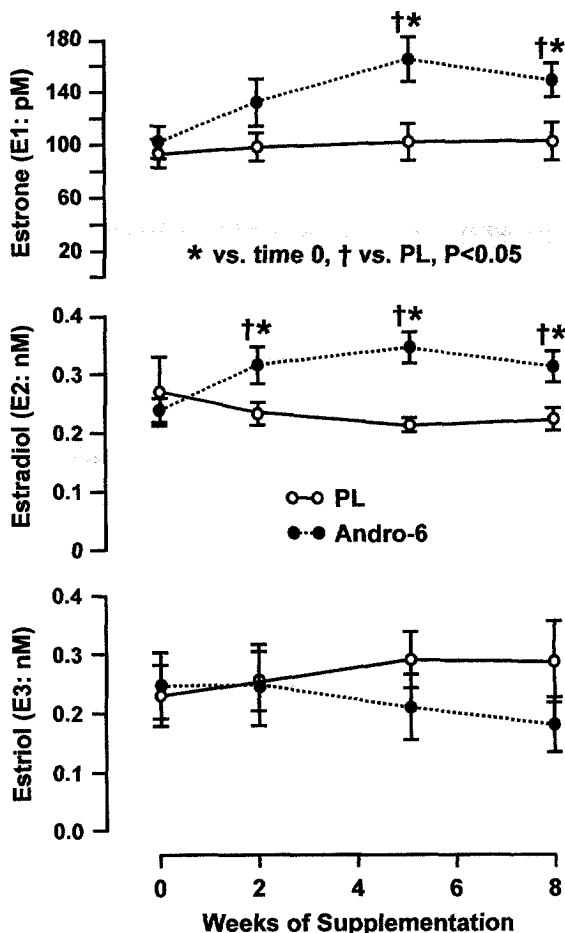


Figure 5 — Serum estradiol, estriol, and estrone concentrations before and during resistance training combined with Andro-6 or placebo (PL) supplementation. Data are means \pm SE for $n = 10$. Note: *Significantly different from Week 0 for Andro-6. †Significantly different from placebo ($p < .05$).

Muscle Histochemistry

Due to the failure of the laboratory freezer and thawing of some of the muscle samples, viable sections were obtained for only 9 PL subjects and 5 ANDRO-6 subjects. The percent of Type I fibers prior to resistance training and supplementation was similar in PL ($44 \pm 6\%$) and ANDRO-6 ($52 \pm 5\%$), and did not change following resistance training in either PL ($44 \pm 5\%$) or ANDRO-6 ($52 \pm 4\%$). The mean cross sectional area of Type I fibers (Figure 7) was not altered with resistance training and supplementation in PL ($3,980 \pm 411$ vs. $4,102 \pm 604 \mu\text{m}^2$) or ANDRO-6 ($3,690 \pm 361$ vs. $3,119 \pm 404 \mu\text{m}^2$). The mean cross sectional area of Type II fibers increased (significant main effect; $p < .05$) in both PL ($5,271 \pm 485$ vs. $5,728 \pm 451 \mu\text{m}^2$) and ANDRO-6 ($3,884 \pm 408$ vs. $4,380 \pm 572 \mu\text{m}^2$).

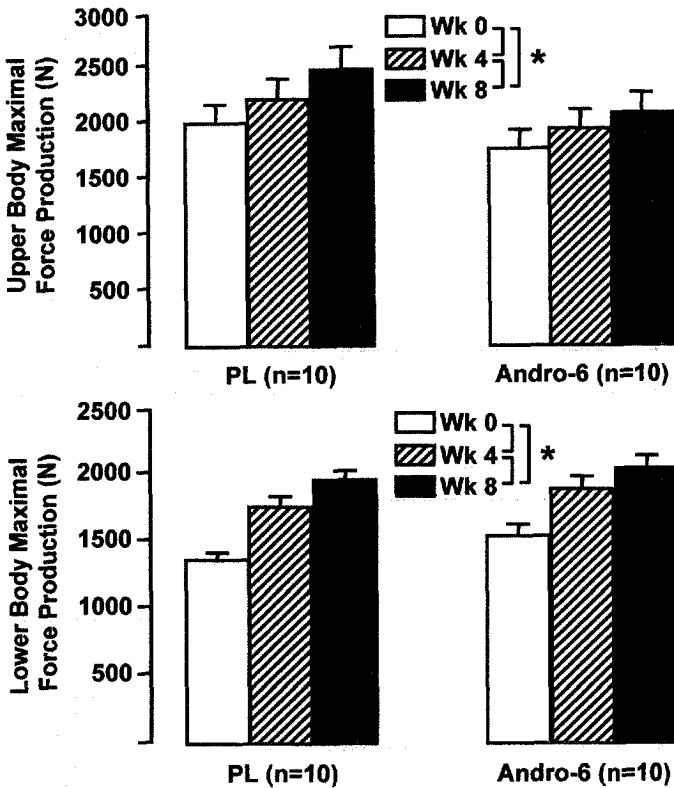


Figure 6 — Upper and lower body strength before and after resistance training combined with Andro-6 or placebo (PL) supplementation. Supplements were administered during Weeks 1–2, 4–5, and 7–8. Upper body strength was calculated as the sum of 1-RM for bench press, shoulder press, and latissimus dorsi pulldown. Lower body strength was calculated as the sum of knee extension, right and left knee flexion. Data are means \pm SE for $n = 10$. Note: *Significantly different from before resistance training (main effect; $p < .05$).

Anthropometric Data

There were no significant differences between ANDRO-6 and PL in the changes in body composition observed as a consequence of resistance training (Table 1). Significant increases in circumferences occurred for the biceps, shoulder, and chest sites (main effect; $p < .05$), while the abdominal, waist, hip, and gluteal circumferences decreased during resistance training in both ANDRO-6 and PL (main effect; $p < .05$). Additionally, the resistance training program (main effect; $p < .05$) reduced the percent body fat in both groups.

Clinical Blood Chemistry

Serum high-density lipoprotein cholesterol (HDL-C) declined by 12% from baseline in ANDRO-6 at Week 2, and remained depressed for the remainder of the 8 weeks (p

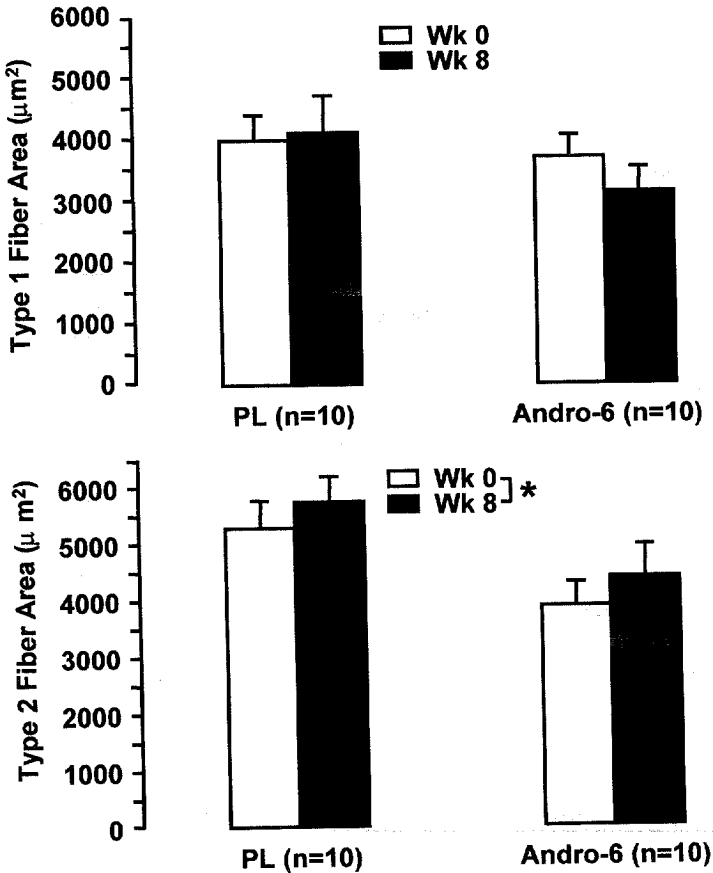


Figure 7 — Mean cross sectional areas of Type I and Type II muscle fibers from the vastus lateralis muscle before and after resistance training combined with Andro-6 ($n = 5$) or placebo (PL, $n = 9$) supplementation. Data are means \pm SE. Note: *Significantly different from before resistance training (main effect; $p < .05$).

$< .05$; Table 2). The 8-week period of training and supplementation did not affect serum concentrations of total cholesterol, LDL cholesterol, VLDL cholesterol, or triglycerides in either treatment group. Serum concentrations of liver function enzymes and red blood cell status were unaltered by training or supplementation (Table 3).

Dietary Control

Total dietary energy ($10,128 \pm 184$ vs. $9,983 \pm 214$ kJ/day), or diet composition for carbohydrate ($50 \pm 1\%$ vs. $51 \pm 1\%$) or protein ($20 \pm 1\%$ vs. $18 \pm 1\%$) intake did not differ for ANDRO-6 and PL treated groups prior to each OGTT. The total dietary energy intake is somewhat lower than expected, possibly due to under-reporting of food weight or volume by the subjects. However, comparison of the glucose and

Table 2 Blood Lipid Data During 8 Weeks of Resistance Training and Supplementation

Variable	Week	PL (n = 10)	ANDRO-6 (n=10)
Triglycerides	0	116.2 ± 26.6	150.1 ± 26.2
	2	110.5 ± 26.2	172.1 ± 31.6
	5	118.7 ± 23.3	142.7 ± 30.0
	8	96.7 ± 22.6	144.9 ± 16.5
LDL-C	0	95 ± 7	86 ± 9
	2	94 ± 8	84 ± 8
	5	105 ± 8	92 ± 10
	8	101 ± 8	93 ± 11
VLDL-C	0	23 ± 5	30 ± 5
	2	22 ± 5	34 ± 6
	5	23 ± 5	28 ± 6
	8	19 ± 5	29 ± 3
HDL-C	0	39 ± 2	42 ± 3
	2	38 ± 3	37 ± 4*
	5	39 ± 3	36 ± 3*
	8	39 ± 3	36 ± 3*

Triglyceride and cholesterol concentrations are expressed as $\text{mg} \cdot \text{dl}^{-1}$. Values are mean \pm SE.

*Significantly different from Wk 0 for ANDRO-6 ($p < .05$).

insulin responses in ANDRO-6 and PL is appropriate, since subjects reproduced the diet prior to the OGTT in the trained state. In addition, subjects were instructed throughout the study to maintain their typical dietary regimen, and all indicated that their dietary practices did not change during the course of the study.

Glucose Tolerance and Plasma Insulin Response

The 8 weeks of resistance training and supplementation resulted in no significant alteration in the incremental area under the glucose curve (Figure 8) for PL or ANDRO-6. Eight weeks of resistance training and supplementation resulted in significant attenuation in the insulin area under the curve for PL and ANDRO-6, which was independent of treatment (main effect; $p < .05$). To provide information regarding the effect of training and supplementation on insulin sensitivity, the product of the incremental glucose and insulin areas (IG Index) was calculated. The IG Index ($\text{mM} \cdot \text{min} \cdot \text{pM} \cdot \text{min} \cdot 10^6$) was reduced by resistance training (main effect; $p < .05$) and did not differ in ANDRO-6 and PL before (ANDRO-6: 18.5 ± 4.5 ; PL: 12.9 ± 2.5) or after (ANDRO-6: 15.9 ± 3.6 ; PL: 11.9 ± 5.4) training and supplementation. Although the incremental area under the insulin curve and the IG Index before training and supplementation tended to be higher in ANDRO-6 compared to PL, this was the result of the extremely high incremental insulin area of one subject

Table 3 Liver Function Enzyme Levels During 8 Weeks of Resistance Training and Supplementation

Variable	Week	PL (n = 10)	ANDRO-6 (n=10)
SGOT	0	19 ± 2	19 ± 2
	2	25 ± 4	20 ± 2
	5	27 ± 4	20 ± 2
	8	24 ± 3	24 ± 2
LDH	0	132 ± 7	133 ± 7
	2	132 ± 7	132 ± 8
	5	138 ± 7	129 ± 7
	*8	143 ± 2	152 ± 5
GGT	0	19 ± 4	24 ± 3
	2	19 ± 5	22 ± 1
	5	25 ± 9	25 ± 3
	8	20 ± 5	22 ± 4
SGPT	0	22 ± 5	19 ± 2
	2	27 ± 7	21 ± 3
	5	34 ± 7	21 ± 3
	8	24 ± 7	21 ± 3

Note. SGOT: glutamate-oxaloacetate transaminase; LDH: lactate dehydrogenase; GGT: gamma-glutamyl transpeptidase; SGPT: glutamate-pyruvate transaminase. Liver enzyme activities are expressed as IU · L⁻¹. Values are means ± SE.

*Significantly different from other weeks for all groups (main effect; $p < .05$)

(118.4 pM · min · 10³), and the mean difference between groups did not approach significance. Removal of this subject's data from the statistical analysis did not affect the results and are therefore included in Figure 8.

Discussion

Ingestion of Andro-6 did not cause short term or long term increases in serum testosterone concentrations in young men, in spite of increased serum androstenedione concentrations. Although many advertisements cite the German patent application (17) as evidence for the efficacy of androstenedione ingestion, the patent application for androstenedione is difficult to evaluate, as it presents no data, and the age, sex, or hormonal status of the subjects tested are not described. Moreover, we have recently demonstrated that acute and chronic androstenedione supplementation does not alter serum testosterone concentrations in young healthy males (21). The present findings confirm these results and suggest that the addition of DHEA and the herbal extracts does not increase serum testosterone concentrations or prevent aromatization of the ingested androgens.

Dehydroepiandrosterone has also been claimed to increase serum testosterone levels. While previous reports have found increased serum testosterone in

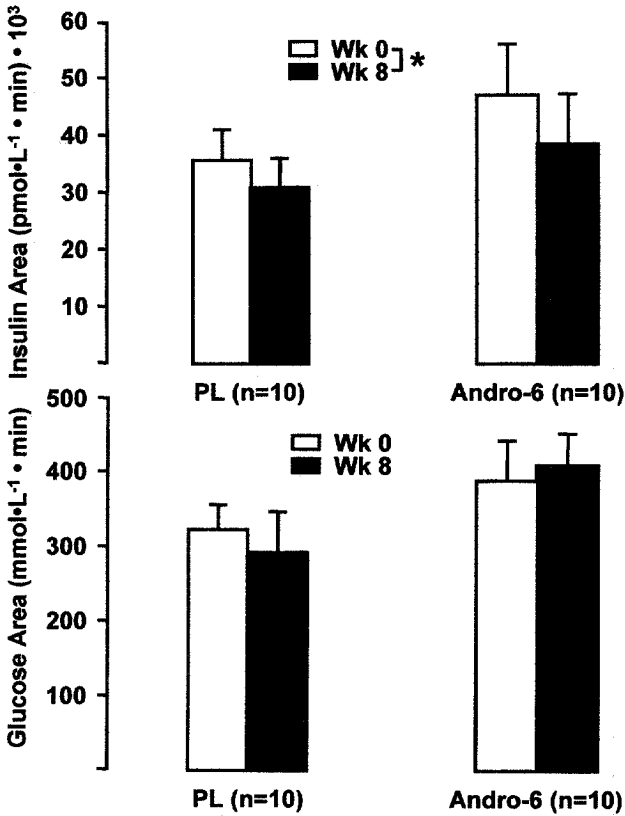


Figure 8 — Incremental area above baseline under glucose and insulin response curve during 75-g oral glucose tolerance tests before and after resistance training combined with Andro-6 or placebo (PL) supplementation. Supplements were administered during Weeks 1–2, 4–5, and 7–8. Data are means \pm SE for $n = 10$. Note: *Significantly different from before resistance training for both treatment groups (main effect; $p < .05$).

women following ingestion of DHEA (25, 29, 30), DHEA ingestion does not increase serum testosterone in men (7, 29, 30, 31). ANDRO-6 ingestion did not produce a dissimilar change in serum testosterone concentrations to previous findings with DHEA and androstenedione administration in men, thus indicating no benefit to combining these androgens in a supplementation regimen.

The use of exogenous anabolic steroids can cause reduced endogenous testosterone production, due to the reduced production of LH and FSH (6, 28). In contrast, ingestion of androstenedione and DHEA do not change serum LH and FSH levels (7, 21), probably due to their weakness as anabolic steroids (34). While *Tribulus terrestris* is advertised to increase serum LH concentrations, the present results suggest that the inclusion of *Tribulus terrestris* in ANDRO-6 does not alter hypothalamic-pituitary function. Ingestion of ANDRO-6 resulted in increased serum estradiol concentrations at Week 2, Week 5, and Week 8 along with increased

serum estrone at Weeks 5 and 8. The increase in serum estrogen levels with ANDRO-6 ingestion is similar to the serum estrogen response we previously reported with androstenedione ingestion (21). Previous research indicates that ingestion of 150 mg/day DHEA does not increase serum estrogen concentrations in men (7, 29, 30). Therefore, the increased serum estradiol and estrone concentrations are likely due to aromatization of the ingested androstenedione, as previously reported (21). This increased formation of estrogens occurred despite the inclusion of I3C and chrysin in ANDRO-6. The dosages of I3C shown to effectively reduce serum estrogen concentrations ($500 \text{ mg} \cdot \text{day}^{-1}$ or $6\text{--}7 \text{ mg} \cdot \text{kg}^{-1} \text{ body weight} \cdot \text{day}^{-1}$; ref. 26, 27) are higher than the dose of I3C ($300 \text{ mg} \cdot \text{day}^{-1}$) included in ANDRO-6. Therefore, it is possible that a higher dose of I3C will prevent aromatization of ingested androstenedione.

There has been little research on chrysin as an aromatase inhibitor, and the dose of chrysin necessary for aromatase inhibition has not been identified, making it difficult to determine the value of the dose of chrysin included in ANDRO-6. While chrysin has been shown to competitively inhibit aromatase, the K_i for chrysin using androstenedione as a substrate is 260 nmol (20), which is much larger than the K_m of 24 nMol for aromatase (12), suggesting that the concentrations of androstenedione found with ANDRO-6 ingestion favor aromatization.

Ingestion of saw palmetto extract has been shown to inhibit 5α -reductase in men (10). However, saw palmetto has not been tested in the presence of ingested androgens, making the effectiveness of the saw palmetto extract in ANDRO-6 difficult to predict. Unfortunately we were unable to obtain measurements of serum DHT concentrations and are therefore unable to provide insight into the effectiveness of saw palmetto extract with ingested androgens.

The increases in muscle strength and muscle fiber area found in the current study demonstrate the effectiveness of the resistance training protocol used. The finding of increased strength with no differences between treatment groups in the current study indicates that ingestion of ANDRO-6 is not effective in promoting muscular strength during resistance training in young males. The current finding of no effect of ANDRO-6 supplementation on muscle strength also indicates that increased serum androstenedione does not enhance muscle strength gains, likely due to the weakness of androstenedione as an anabolic-androgenic steroid (34).

The effect of DHEA ingestion on body composition in humans is equivocal (7, 29, 30). Nestler et al. (31) observed that consumption of 1600 mg DHEA per day for 28 days decreased body fat and increased muscle mass in young men. In contrast, Welle et al. (37) observed that the intake of 1600 mg DHEA per day for 4 weeks did not alter energy or protein metabolism, body weight or two indices of lean body mass (total body water and total body potassium). In the present study, the decreased body fat and increased lean body mass observed with resistance training was unaffected by daily supplementation with ANDRO-6, which contained 150 mg DHEA. Our previous research with androstenedione ingestion in young men indicates that ingesting 300 mg androstenedione per day during resistance training did not enhance changes in body composition during resistance training. Although the effect of higher doses of DHEA and androstenedione on body composition are unclear, these data indicate that ingestion of DHEA and androstenedione in the dosages contained in ANDRO-6 are insufficient to promote changes in body composition and strength beyond those normally associated with resistance training in healthy young males.

The use of anabolic steroids has been shown to cause insulin resistance (8, 13). Since androstenedione is a weaker androgenic hormone than testosterone (34), it is likely that any deleterious effect of androstenedione on insulin action was more than compensated for by the improved insulin action brought about by resistance training. Previous research has found no effect of DHEA ingestion on glucose tolerance in men (7, 31, 37), while the effect of androstenedione, saw palmetto, I3C, chrysin and Tribulus terrestris ingestion on glucose tolerance has not been previously reported. The current research found reduced plasma insulin levels during an OGTT following resistance training in both groups, in spite of increased levels of serum androstenedione with ANDRO-6 supplementation, suggesting that these compounds do not significantly alter insulin action or secretion.

Lowered blood HDL-C cholesterol concentrations with androgenic steroid usage occurs due to an increase in hepatic triacylglycerol lipase activity followed by a reduction in the HDL2 subfraction (19). The 12% reduction in blood HDL-C levels with ANDRO-6 supplementation in the current study is identical to the reduction observed with androstenedione ingestion alone (21), and less than the 50% or greater reduction found in other studies using anabolic steroids (9, 18). Since DHEA has not previously reduced serum HDL-C concentrations in men (7, 29, 30, 31, 37), the lowered HDL-C levels seen with ANDRO-6 supplementation are likely due to the ingestion of androstenedione (21). The 12% reduction in serum HDL-C cholesterol found with ANDRO-6 ingestion reflect a 10–15% increase in the risk for heart disease (16), indicating a potential for serious health consequences with ANDRO-6 use.

The increased serum estradiol concentrations observed during the 6 hr after ingestion of both placebo and Andro-6 is consistent with the previously observed circadian variations observed in men (1). However, the finding of chronically increased serum estrogen concentrations with ANDRO-6 supplementation, in spite of the inclusion of I3C and chrysin in ANDRO-6, suggests that ANDRO-6 poses similar health risks to androstenedione ingestion (21). Increased serum estrogen levels in men have been linked to prostate hypertrophy (4), pancreatic cancer (13), cardiovascular disease (32), and gynecomastia (23). Elevated serum androstenedione concentrations may also be associated with some health risks, since elevated serum androstenedione levels may increase the risk of pancreatic (3) and prostate cancer (4). Furthermore, androstenedione has recently been demonstrated to be equally potent to testosterone in terms of its neural effects (36).

In summary, the addition of DHEA, saw palmetto, Tribulus terrestris, chrysin, and indole-3-carbinol to a supplement containing androstenedione does not enhance serum testosterone concentrations, changes in body composition, or strength associated with resistance training. These compounds do not prevent the increased formation of estrogens or decreased HDL-C observed with androstenedione supplementation.

References

1. Ahokoski, O., A. Virtanen, R. Huupponen, H. Scheinin, E. Slamminen, V. Kairusto, and K. Irjala. Biological day-to-day variation and daytime changes of testosterone, follitropin, lutropin and oestradiol-17beta in healthy men. *Clin. Chem. Lab. Med.* 36:485-491, 1998.
2. American College of Sports Medicine. *Guidelines for Exercise Testing and Prescription* (5th ed.). Media, PA: Williams and Wilkins, 1995.

3. Andersson, S.O., H.O. Adami, R. Bergstrom, and L. Wide. Serum pituitary and sex steroid hormone levels in the etiology of prostatic cancer: a population-based case-controlled study. *Br. J. Cancer* 68:97-102, 1993.
4. Barrett-Connor, E., C. Garland, J.B. McPhillips, K.T. Khaw, and D.L. Wingard. A prospective population based study of androstenedione, estrogens, and prostatic cancer. *Cancer Res.* 50:169-173, 1990.
5. Bergstrom J. Muscle electrolytes in man. *Scand. J. Clin. Lab. Invest.* 17(Suppl.):68-110, 1967.
6. Bhasin, S., T.W. Storer, N. Berman, C. Callegari, B. Clevenger, J. Phillips, T.J. Bunnell, R. Tricker, A. Shirazi, and R. Casaburi. The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. *N. Engl. J. Med.* 335:1-7, 1996.
7. Brown, G.A., M.D. Vukovich, R.L. Sharp, T.A. Reifenrath, K.A. Parsons, and D.S. King. Effect of oral dehydroepiandrosterone on serum testosterone and adaptations to resistance training. *J. Appl. Physiol.* 87:2274-2283, 1999.
8. Cohen, J.C., and R. Hickman. Insulin resistance and diminished glucose tolerance in powerlifters ingesting anabolic steroids. *J. Clin. Endocrinol. Metab.* 64:960-963, 1987.
9. Costill, D.L., D.R. Pearson, and W.J. Fink. Anabolic steroid use among athletes: changes in HDL-C levels. *Phys. Sportsmed.* 12:113-117, 1984.
10. DiSilverio, F., G. D'Eramo, C. Lubrano, G.P. Flammia, A. Sciarra, E. Palma, M. Caponera, and F. Sciarra. Evidence that Serona repens extract displays an antiestrogenic activity in prostatic tissue of benign prostatic hypertrophy patients. *Eur. Urol.* 21:309-314, 1992.
11. Freed, D.L., A.J. Banks, D. Longson, and D.M. Burley. Anabolic steroids in athletics: crossover double-blind trial in weightlifters. *BMJ.* 31:471-473, 1975.
12. Forney, J., P.L. Milewich, G.T. Chen, J.L. Garlock, B.E. Schwarz, C.D. Edman, and P.C. MacDonald. Aromatization of androstenedione to estrone by human adipose tissue in vitro. *J. Clin. Endocrinol. Metab.* 53:192-199, 1981.
13. Fyssas, I., K.N. Syrigos, M.M. Konstandoulakis, S. Papadopoulos, N. Milingos, M. Anapliotou, J. Waxman, and B.C. Golematis. Sex hormones in the serum of patients with pancreatic adenocarcinoma. *Horm. Metab. Res.* 29:115-118, 1997.
14. Godsland, I.F., Shennan, N.M., and V. Wynn. Insulin action and dynamics modeled in patients taking the anabolic steroid methandienone (Dianabol). *Clin. Sci.* 71:665-673, 1986.
15. Goldman, H.I., and M.R. Becklake. Respiratory function tests. *Am. Rev. Tuberc. Pulmon. Dis.* 79:457-467, 1959.
16. Gordon, D.J., J.L. Probstfield, R.J. Garrison, J.D. Neaton, W.P. Castelli, J.D. Knoke, D.R. Jacobs Jr., S. Bangdiwala, and H.A. Tyroler. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation* 79:8-15, 1989.
17. Hacker, R., and C. Mattern. German Patent DE 42 14953 C2 1995.
18. Hurlley, B.F., D.R. Seals, J.M. Hagberg, A.C. Goldberg, S.M. Ostrove, J.O. Holloszy, W.G. Wiest, and A.P. Goldberg. High-density-lipoprotein cholesterol in bodybuilders v. powerlifters. *JAMA.* 252:507-513, 1984.
19. Kantor, M.A., A. Bianchini, D. Bernier, S.P. Saday, and P.D. Thompson. Androgens reduce HDL2-cholesterol and increase hepatic triglyceride lipase activity. *Med. Sci. Sports Exerc.* 17:462-465, 1985.
20. Kellis, J.T., Jr., and L.E. Vickery. Inhibition of human estrogen synthetase (aromatase) by flavones. *Science* 225:1032-1034, 1984.
21. King D.S., R.L. Sharp, M.D. Vukovich, G.A. Brown, T.A. Reifenrath, N.L. Uhl, and K.A. Parsons. Effect of oral androstenedione on serum testosterone and adaptations to resistance training in young men. *JAMA.* 281:2020-2028, 1999.
22. Lenders, J.W., P.N. Demacker, J.A. Vos, P.L. Jansen, A.J. Hoitsma, A. van't Laar, and T. Thien. Deleterious effects of anabolic steroids on serum lipoproteins, blood pressure, and liver function in amateur body builders. *Int. J. Sports Med.* 9:19-23, 1988.

23. Lewin, M.L. Gynecomastia: the hypertrophy of the male breast. *J. Clin. Endocrinol.* 1:511-514, 1941.
24. Longcope, C. Dehydroepiandrosterone metabolism. *J. Endocrinol.* 150(Suppl.):S125-S27, 1996.
25. Mahesh, V.B., and R.B. Greenblatt. The in vivo conversion of dehydroepiandrosterone and androstenedione to testosterone in the human. *Acta Endocrinol.* 41:400-406, 1962.
26. Michnovicz, J.J., and H.L. Bradlow. Induction of estradiol metabolism by dietary indole-3-carbinol in humans. *J. Natl. Cancer Inst.* 82:947-949, 1990.
27. Michnovicz, J.J., and H.L. Bradlow. Altered estrogen metabolism and excretion in humans following consumption of indole-3-carbinol. *Nutr. Cancer* 65:59-66, 1991.
28. Mooradian, A.D., J.E. Morley, and S.G. Korenman. Biological actions of androgens. *Endocr. Rev.* 8:1-28, 1987.
29. Morales, A.J., R.H. Haubrich, J.Y. Hwang, H. Asakura, and S.S.C. Yen. The effect of six months treatment with a 100 mg daily dose of dehydroepiandrosterone (DHEA) on circulating sex steroids, body composition and muscle strength in age-advanced men and women. *Clin. Endocrinol.* 49:421-432, 1998.
30. Morales, A.J., J.J. Nolan, J.C. Nelson, and S.S. Yen. Effects of a replacement dose of dehydroepiandrosterone in men and women of advancing age. *J. Clin. Endocrinol. Metab.* 78:1360-1367, 1994.
31. Nestler, J.E., C.O. Barlaschini, J.N. Clore, and W.G. Blackard. Dehydroepiandrosterone reduces serum low density lipoprotein levels and body fat but does not alter insulin sensitivity in normal men. *J. Clin. Endocrinol. Metab.* 66:57-61, 1988.
32. Phillips, G.B., B.H. Pinkernell, and T.Y. Jing. The association of hyperestrogenemia with coronary thrombosis in men. *Arterioscler. Thromb. Vac. Biol.* 16:1383-1387, 1996.
33. Siri, W.E. Body composition from fluid spaces and density. In: *Techniques for Measuring Body Composition*, J. Brozek and R. Henschel (Eds.). Washington, DC: National Academy of Science, 1961, pp. 223-244.
34. Sommerville, I.F., and W.P. Collins. Indices of androgen production in women. In: *Advances in Steroid Biochemistry and Pharmacology, Volume 2*, M.H. Briggs (Ed.). New York and London: Academic Press, 1971, p. 266.
35. Sultan, C., A. Terraza, C. Devillier, E. Carilla, M. Briley, C. Loire, and B. Descomps. Inhibition of androgen metabolism and binding by a liposterolic extract of "Serona repens B" in human foreskin fibroblasts. *J. Steroid Biochem.* 20:515-519, 1984.
36. Villalba, C., C.J. Auger, and G.J. DeVries. Androstenedione effects on the vasopressin innervation of the rat brain. *Endocrinology* 140:3383-3386, 1999.
37. Welle, S., R. Jozefowicz, and M. Statt. Failure of dehydroepiandrosterone to influence energy and protein metabolism in humans. *J. Clin. Endocrinol. Metab.* 71:1259-1264, 1990.

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