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January 31, 1997

TO: Thomas J. Brorby, Esq.  
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RE: Chronology of DHEA studies in my laboratory

FROM: Samuel S.C. Yen, M.D., D.Sc. 

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Investigational drug of DHEA (IND #32,554) was granted by the FDA on December 29, 1988.

- Our first clinical trial to determine endocrine-metabolic impacts of a pharmacological dose of DHEA (1600mg/day) for 4 weeks duration in postmenopausal women was published in Journal of Clinical Endocrinology & Metabolism 71:696-704, 1990. In this study, several important unfavorable effects were demonstrated (see reprint).
- The second clinical trial using replacement dose of DHEA (50mg/day) both orally and sublingually in age-advanced men and women was initiated in 1992. The results of this study have demonstrated several beneficial effects in both age-advanced men and women. More important is the absence of significant adverse effects; Journal Clinical Endocrinology & Metabolism 78:1360-1367, 1994.
- The third clinical trial was initiated in 1993 with the aim to assess the biological endpoints of DHEA at 100mg daily dose. Changes in body composition and muscle strength were evident in age-advanced men but not women - the potential gender differences was considered and remain to be elucidated (see below). The findings were published in the symposium on DHEA and Aging; New York Academy of Sciences 774:128-142, 1995.
- The fourth clinical trial concerning the potential beneficial effects of DHEA (50mg/day) in immune function in age-advanced men was initiated in 1994. Activation of T-lymphocytes and natural killer (NK) cells occurred as evidence by

significant increased of IL-2, IL-2 receptor and soluble IL-2 receptor, and by a stimulatory effect of DHEA on cytolytic effects of NK cells. The results of this study has been published in Journal of Gerontology: Biological Sciences and Medical Sciences 52A:M2-M7, 1997.

All above cited studies except the pharmacological dose (1990) of DHEA were without significant adverse effects.

- Current clinical trials include 1) the role of DHEA in the treatment of Addison's disease and 2) the effect of hormone replacements may blunt the beneficial effects of DHEA in postmenopausal women.

Encl (reprints x 4)  
cc: Irene Chow, Ph.D. (Genelabs)

## The Effects of Oral Dehydroepiandrosterone on Endocrine-Metabolic Parameters in Postmenopausal Women\*

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**ABSTRACT.** To discern the pharmacological effects of dehydroepiandrosterone (DHEA) in older women with low endogenous DHEA and DHEA sulfate (DS), 1600 mg/day (in four divided doses) were administered orally to six postmenopausal women for 28 days in a double blind placebo-controlled cross-over study. Serum concentrations of androgens after the first 400-mg dose of DHEA increased rapidly and reached a maximum at 180–240 min, resulting in increases over baseline of 6-fold for DHEA ( $5.8 \pm 2.1$  to  $28.8 \pm 5.5$  nmol/L), 12-fold for DS ( $3.0 \pm 1.6$  to  $28.2 \pm 4.6$   $\mu$ mol/L) and androstenedione ( $1.4 \pm 0.3$  to  $19.5 \pm 9.8$  nmol/L), 2.5-fold for testosterone ( $0.7 \pm 0.1$  to  $2.2 \pm 0.6$  nmol/L), and 15-fold for dihydrotestosterone ( $0.2 \pm 0.06$  to  $2.73 \pm 1.0$  nmol/L), but estrone, estradiol, and sex hormone-binding globulin (SHBG) were unchanged.

Assessment at weekly intervals revealed a further increase in all androgens which was maximal at 2 weeks and remained markedly elevated, although it declined somewhat by 4 weeks. The increments observed after 2 weeks of DHEA administration reached 15-fold for DHEA ( $71.9 \pm 14.2$  nmol/L), 9-fold for testosterone ( $6.5 \pm 1.7$  nmol/L), and 20-fold for DS ( $65.1 \pm 14.9$  nmol/L), androstenedione ( $30.5 \pm 11.5$  nmol/L), and dihydrotestosterone ( $3.8 \pm 1.5$  nmol/L). Both estrone and estradiol showed a progressive increase to 2-fold the basal value at 4 weeks. Integrated SHBG and thyroid binding globulin levels decreased ( $P < 0.05$ ) during DHEA treatment. However, LH, FSH, body weight, and percent body fat, as measured by underwater weighing, were unaltered during the 4-week experiment.

A marked decline of 11.3% ( $P < 0.05$ ) in serum cholesterol and 20.0% ( $P < 0.05$ ) in high density lipoprotein noted within the first week of DHEA administration persisted for the 28-day period and was accompanied by a nonsignificant downward trend in low density lipoprotein, very low density lipoprotein, and triglycerides. Peak insulin levels during the 3-h oral glucose tolerance test were significantly higher ( $P < 0.05$ ) after the 28 days of DHEA ( $1126 \pm 165$  vs.  $746 \pm 165$  pmol/L) and were accompanied by a 50% increase in the integrated insulin response ( $P < 0.01$ ) without a significant change in fasting glucose insulin or glucose-6-phosphate dehydrogenase values.

These data show that oral DHEA is rapidly absorbed, and a prompt conversion to DS as well as all potent androgens and estrogens occurs with a sustained effect for the 28-day duration of treatment. Thus, in postmenopausal women there appears to be abundant and effective enzymatic systems for the biotransformation of DHEA to C-19 and C-18 sex steroids. Further, pharmacologically imposed DHEA in postmenopausal women induced insulin resistance, altered cholesterol and the high to low density lipoprotein ratio, and decreased circulating SHBG and thyroid binding globulin concentrations. Our findings, which contrast sharply with those of a previous experiment in young men, appear to result from the induction of a highly androgenic state and its impact on several endocrine-metabolic parameters in older postmenopausal women. (*J Clin Endocrinol Metab* 71: 696–704, 1990)

**C**IRCULATING levels of dehydroepiandrosterone (DHEA) and its sulfate ester (DS) at birth are higher than the upper limits of the adult range (1, 2) and fall rapidly until adrenarche (1), when a marked increase occurs for both sexes (2, 3). Concentrations of DHEA

and DS peak during the third decade, reaching levels at least 15-fold that of prepubertal nadir, and thereafter progressively decline to very low levels at senescence (4). These dramatic changes in adrenal secretion of androgen during the lifespan without significant alterations of cortisol secretion may be explained in part by an increase followed by a decrease in 17,20-desmolase activity in the adrenal cortex (5–8). The physiological significance of the “switch on” and “switch off” of DHEA and DS production from young adulthood to aging, respectively, is unclear.

Administration of DHEA to experimental animals has shown beneficial effects on endocrine-metabolic parameters, enhanced immunoprotective functions, and reduced carcinogenesis. DHEA prevents diabetes in genet-

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ically diabetic (9) and obese (10) mice. Tissue sensitivity to insulin is increased in aged normal mice (11), and weight gain is slowed without changing food intake in younger animals (12). DHEA prevents the increases in cholesterol in rats rendered hypothyroid with propylthiouracil (13) and retards atherosclerotic plaque formation in rabbit aorta (14). Moreover, in rodents, DHEA increases the lifespan (15), restrains autoimmune disease (15, 16), up-regulates immune systems (17-19), and inhibits the development of spontaneous breast cancer (20). DHEA treatment decreased levels of glucose-6-phosphate dehydrogenase (G6PD) enzyme activity in both mouse embryo fibroblast (21) and human lymphocyte and skin fibroblast cultures (22) and was associated with an increased resistance to cellular transformation by some carcinogens (22, 23). The importance of these experimental findings, both *in vivo* and *in vitro*, is underscored by epidemiologic data that low DHEA levels are correlated with increased cardiovascular morbidity in men (24), breast cancer in women (25), and the decline in immune competence (26).

In humans, oral administration of DS results in both androgenic and estrogenic manifestations (27), which may account for its myriad effects. Recently, Nestler *et al.* (28) have reported that the oral administration of DHEA at a dose of 1600 mg/day to 22- to 25-yr-old men for 28 days resulted in lowering of cholesterol and low density lipoprotein (LDL) cholesterol without changes in other lipid parameters and glucose disposal. Body fat also decreased, with a tendency for a greater response in more obese individuals. In contrast, using a depot injection of 200 mg DHEA enanthate, an earlier uncontrolled study in young ovariectomized women (29) found a decrease in total cholesterol accompanied by decreased high density lipoprotein (HDL) at 14 and 30 days, with a modest increase in androgen and estrogen levels.

Thus, the implications of DHEA are numerous, but the impact of exogenous DHEA on endocrine-metabolic profiles in aging women with reduced endogenous production of adrenal DHEA has not been studied. We now report the results of oral administration of 1600 mg (four divided doses) of DHEA for 28 days to six obese postmenopausal women in a double blind placebo-controlled cross-over study.

### Materials and Methods

Six postmenopausal women, between the ages of 46-61 yr, were selected for study. All women were 30-50% over ideal body weight (Table 1) by the Metropolitan Life table (30). One woman had undergone bilateral oophorectomy, and the remainder had spontaneous menopause of more than 1-yr duration. Before the study, menopause was confirmed by elevated FSH and LH levels (Table 1). Serum DHEA and DS were significantly lower than those in young women in our laboratory

(DHEA,  $5.8 \pm 2.1$  vs.  $23.8 \pm 6.6$  nmol/L; DS,  $3.0 \pm 1.6$  vs.  $5.2 \pm 1.3$   $\mu$ mol/L). No subject had taken hormone replacement therapy or other prescribed medication for the previous year. Medical illness was excluded by history, physical examination, serum chemistry profile, including renal liver and thyroid panels, urinalysis, and complete blood count.

### Study design

Each woman was randomly assigned to receive DHEA or placebo first. Subjects received 400 mg, orally, DHEA (or placebo) four times a day for a 28-day period (1600 mg/day). After an intervening 2-week washout period, a cross-over of treatment (DHEA or placebo) for 28 days was accomplished. DHEA was purchased from Sigma Chemical Co. (St. Louis, MO) and packaged in gelatin capsules (200 mg/capsule). This study was approved by the Committee on Investigations Involving Human Subjects of the University of California, San Diego, and informed consent was obtained before the start of the study.

Before the first dose of DHEA (or placebo), a 75-g oral glucose tolerance test (oGTT) was performed after an overnight fast. Serum glucose and insulin values were measured at 0, 30, 60, 120, and 180 min. oGTTs were repeated on the last day of each treatment cycle (day 28). The percent body fat was determined at the start and end of each treatment period.

Serum levels of DHEA, DS, androstenedione (A), testosterone (T), dihydrotestosterone (DHT), estrone (E<sub>1</sub>), estradiol (E<sub>2</sub>), and sex hormone-binding globulin (SHBG) were evaluated before and after the first 400-mg oral dose of DHEA at 0, 30, 60, 120, 180, and 240 min. These variables were determined at weekly intervals throughout the study. In addition, weekly fasting serum glucose, G6PD, and fractional lipid determinations, including total cholesterol, HDL, low density lipoprotein cholesterol (LDL), triglycerides, and very low density lipoprotein cholesterol (VLDL) were obtained. Evaluations of thyroid function [total T<sub>4</sub>, T<sub>3</sub>, T<sub>3</sub> resin uptake (T<sub>3</sub>RU), free T<sub>4</sub>, thyroid binding globulin (TGB), and TSH], liver function (aspartate amino transaminase, alanine amino transaminase, gamma glutamyl transferase, alkaline phosphatase, and total bilirubin), renal function (blood urea nitrogen and creatinine), electrolytes, complete blood count (including red cell indices, white cell differential, and platelet count), and urinalysis were examined weekly. The physical exam was repeated at the start and end of each treatment cycle. Subjects were given 24-h diet recall inventories at random intervals throughout the study to detect differences in caloric intake and percent dietary fat, protein, and carbohydrate between the two treatment cycles.

### Measurements

The percent ideal body weight for each subject was determined by Metropolitan Life table analysis (30). Ponderal index was calculated by determining the height in inches divided by the cubed root of weight in pounds. Percent body fat was determined by underwater weighing methods developed by Behnke *et al.* (31). The formula developed by Siri was used to calculate the percent body fat (32).

Steroid and gonadotropin determinations were performed by

TABLE 1. Anthropomorphic measurements and laboratory characteristics before and after 28 days of treatment with DHEA and placebo in six postmenopausal women

	DHEA		Placebo	
	Day 0	Day 28	Day 0	Day 28
Wt (kg)	80.6 ± 1.7 (75.0-87.2)	82.2 ± 1.9 (77.7-87.7)	81.4 ± 1.8 (75.0-85.0)	82.2 ± 1.7 (76.4-85.9)
% IBW	140 ± 3 (130-150)	143 ± 3 (134-155)	142 ± 2 (134-150)	143 ± 3 (135-155)
Ponderal index	11.4 ± 0.1 (11.4-12.0)	11.5 ± 0.1 (11.1-11.8)	11.6 ± 0.1 (11.3-12.0)	11.5 ± 0.1 (11.1-11.8)
% Body fat	47.3 ± 0.9 (45.7-51.2)	47.3 ± 0.7 (45.7-50.1)	47.5 ± 0.6 (45.9-49.3)	47.3 ± 0.6 (45.9-49.5)
LH (IU/L)	118 ± 20 (76-141)	112 ± 11 (71-183)	107 ± 17 (75-156)	116 ± 19 (81-172)
FSH (IU/L)	118 ± 12 (95-160)	119 ± 10 (87-148)	115 ± 13 (89-151)	113 ± 11 (87-142)
Glucose (fasting; nmol/L)	4.2 ± 0.2 (3.8-4.9)	3.9 ± 0.3 (2.8-4.4)	4.2 ± 0.3 (3.3-4.9)	4.1 ± 0.2 (4.0-4.6)
Insulin (pmol/L)	114 ± 32 (57-287)	118 ± 31 (64-238)	119 ± 35 (57-202)	112 ± 25 (79-281)
G6PD (IU/g hemoglobin)	8.9 ± 1.0 (8.5-9.7)	8.8 ± 0.7 (7.9-9.8)	9.1 ± 0.1 (8.9-9.7)	8.5 ± 0.4 (7.0-10.0)

The range is given in parentheses. IBW, Ideal body weight.

RIA by previously described methods (33, 34). All samples were assayed in duplicate. The sensitivity of the assays were 0.35 nmol/L for DHEA, 0.5  $\mu$ mol/L for DS, 0.2 nmol/L for T, 0.3 nmol/L for A, 0.2 nmol/L for DHT, 29 pmol/L for E<sub>1</sub>, 70 pmol/L for E<sub>2</sub>, 0.3 mIU/L for LH, and 2 IU/L for FSH. The intra- and interassay coefficients of variation were, respectively, 8% and 9% for DHEA, 5% and 10% for DS, 7% and 12% for A, 7% and 9% for T, 8% and 11% for DHT, 11% and 12% for E<sub>1</sub>, 9% and 10% for E<sub>2</sub>, 7% and 10% for LH, and 8% and 12% for FSH.

SHBG was measured by previously described methods using incubation of DHT with serum followed by ammonium sulfate precipitation (35). Glucose determinations were made using a Beckman glucose analyzer (Beckman, Fullerton, CA) immediately after sample collection. Insulin was assayed by a modification of the technique of Kuzuya *et al.* (36). These modifications yielded an assay sensitivity of 2.1  $\mu$ U/mL, and intra- and interassay coefficients of variation were 7% and 9%, respectively. G6PD determinations were obtained by a fluorometric assay of NADPH generated by erythrocytes (37) (Central Diagnostic Laboratories, Tarzana, CA).

Total cholesterol and triglycerides were measured by previously described enzymatic methods, and HDL cholesterol was directly determined after sodium phosphotungstate precipitation of other lipids (38). LDL cholesterol was calculated using the formula: LDL = cholesterol - (HDL + VLDL). VLDL was calculated by dividing the triglyceride value by 5. Complete blood counts, liver function studies, thyroid studies, electrolytes, and urinalysis used to monitor subject safety were performed in commercial laboratories (Central Diagnostic Laboratories).

#### Analyses of data

Differences between all steroid hormone concentrations as well as SHBG acutely after the first dose of DHEA and in

weekly determinations were performed by two-way analysis of variance with repeated measures, with factors being subject and treatment, followed by Newman-Keuls multiple range tests. Differences in glucose and insulin after the oGTT and weekly G6PD and glucose determinations were similarly analyzed. Lipid fractions were converted to percent change from baseline values and analyzed by an analogous two-way analysis of variance, followed by Duncan testing. Integrated SHBG levels and integrated insulin and glucose responses during the oGTT before and after DHEA treatment were determined by calculating the area under the response curves by the trapezoid method and compared by paired *t* test. Differences in body weight, percent body fat, thyroid function studies, and gonadotropin levels were compared by analysis of variance. Data are presented for each time point as the mean  $\pm$  SE. For all analyses *P* < 0.05 was considered significant.

## Results

### Acute effects

A prompt and dramatic increase in all serum androgen levels after the first oral dose of 400 mg DHEA was observed (Fig. 1, left panel). Mean ( $\pm$ SE) maximal increments (6-fold) in serum DHEA were seen 180 min after DHEA ingestion (5.8  $\pm$  2.1 to 28.8  $\pm$  5.5 nmol/L). Serum DS rose 10-fold above the basal level at 180 min and 12-fold at 240 min (3.0  $\pm$  1.6 to 28.2  $\pm$  4.6  $\mu$ mol/L). A parallel increase in serum A was evident and reached 12-fold the basal value at 240 min (1.42  $\pm$  0.3 to 19.5  $\pm$  9.8 nmol/L). Serum T levels showed a slower and modest increase (2.5-fold) at 240 min. In contrast, the rise in serum DHT was more robust, with a 4-fold increase seen within 120 min and a further rise to 15-fold at 180 min

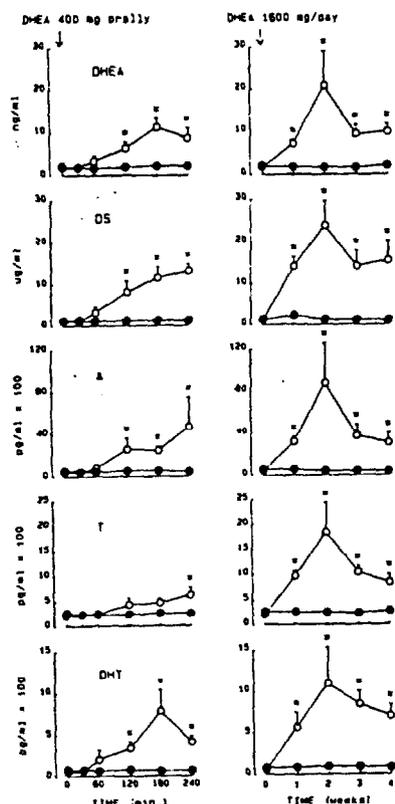


FIG. 1. Serum concentration of androgens obtained within 240 min after the first 400 mg dose of DHEA (left panels) and at weekly intervals during DHEA administration in a daily dose of 1600 mg. O, DHEA; ●, placebo. \*,  $P < 0.05$  compared with basal value. Concentrations are reported in metric units. To convert to System Internationale, multiply by the following factors: DHEA  $\times 3.467 = \text{nmol/L}$ , DS  $\times 2.714 = \text{nmol/L}$ , A  $\times 0.00349 = \text{nmol/L}$ , T  $\times 0.00347 = \text{nmol/L}$ , and DHT  $\times 0.00344 = \text{nmol/L}$ .

( $0.2 \pm 0.06$  to  $2.7 \pm 1.0 \text{ nmol/L}$ ). The increments in androgen levels, expressed as percent change from basal values, are shown in Table 2. No significant changes in serum  $E_1$ ,  $E_2$ , or SHBG levels were observed after the single dose of DHEA. There were no significant altera-

tions in any of the above-mentioned parameters during the placebo treatment.

#### Chronic effect

Daily administration of 1600 mg DHEA (in four divided doses) for 28 days exhibited further elevations in androgen levels, evaluated at weekly intervals before the first morning dose (Fig. 1, right panel). The percent increment for each androgen is given in Table 2. The highest serum values were seen 2 weeks after the start of the experiment for all androgens; mean increments from basal values reached 12-fold for DHEA ( $72.0 \pm 14.2 \text{ nmol/L}$ ), 9-fold for T ( $6.5 \pm 1.7 \text{ nmol/L}$ ), and 20-fold for DS ( $65.1 \pm 14.9 \mu\text{mol/L}$ ), A ( $30.5 \pm 11.5 \text{ nmol/L}$ ), and DHT ( $3.8 \pm 1.5 \text{ nmol/L}$ ). While the weekly values for SHBG were not significantly different, mean integrated serum SHBG concentrations were lower ( $P < 0.05$ ) during the 28 days of DHEA treatment compared with those after the placebo treatment ( $43 \pm 5$  vs.  $57 \pm 9 \text{ nmol/L} \cdot \text{week}$ ; Fig. 2). A progressive rise in serum  $E_1$  and  $E_2$  levels became apparent during the course of the 28-day experiment, with mean  $E_1$  and  $E_2$  values increasing 2–3-fold above basal values ( $E_1$ ,  $58.7 \pm 11.0$  to  $167.4 \pm 66.6 \text{ pmol/L}$ ;  $E_2$ ,  $36.7 \pm 3.7$  to  $121.1 \pm 25.7 \text{ pmol/L}$ ), corresponding to maximal percent changes of  $214 \pm 67\%$  and  $181 \pm 29\%$  for  $E_1$  and  $E_2$ , respectively (Fig. 3). Despite the marked increases in androgens and estrogens, gonadotropin levels showed no discernible changes throughout the experiment (Table 1). With the placebo treatment, none of the above-mentioned parameters was altered.

#### Lipids and glucose metabolism

Serum cholesterol declined  $11.3 \pm 3.3\%$  ( $P < 0.05$ ) within the first week of DHEA treatment; this was maintained throughout the 4 weeks of treatment (Fig. 4) and was accompanied by a  $20.0 \pm 4.9\%$  decline ( $P < 0.05$ ) in HDL cholesterol within the first week, which remained 20–30% lower for the duration of the study.

TABLE 2. Percent change from basal levels in androgens and SHBG after the first oral dose of 400 mg DHEA and at weekly intervals during daily administration of 1600 mg/day in six postmenopausal women

Time	DHEA	DS	A	T	DHT	SHBG
<b>Acute</b>						
30 min	$15 \pm 16$	$2 \pm 2$	$12 \pm 14$	$3 \pm 6$	$12 \pm 11$	$-1 \pm 2$
60 min	$126 \pm 98^*$	$164 \pm 112^*$	$213 \pm 198^*$	$24 \pm 12^*$	$314 \pm 247^*$	$-6 \pm 3$
120 min	$253 \pm 65^*$	$525 \pm 159^*$	$526 \pm 210^*$	$110 \pm 52^*$	$585 \pm 182^*$	$-3 \pm 2$
180 min	$643 \pm 178^*$	$1026 \pm 251^*$	$676 \pm 261^*$	$150 \pm 49^*$	$1570 \pm 601^*$	$1 \pm 4$
240 min	$452 \pm 187^*$	$1386 \pm 383^*$	$1332 \pm 887^*$	$236 \pm 88^*$	$748 \pm 186^*$	$2 \pm 5$
<b>Daily</b>						
1 week	$386 \pm 112^*$	$1385 \pm 337^*$	$894 \pm 256^*$	$447 \pm 98$	$928 \pm 283$	$-32 \pm 6$
2 weeks	$1338 \pm 635^*$	$2993 \pm 1146^*$	$3378 \pm 1985^*$	$1017 \pm 458^*$	$2101 \pm 946^*$	$-31 \pm 14$
3 weeks	$527 \pm 181^*$	$1942 \pm 910^*$	$1109 \pm 429^*$	$488 \pm 114^*$	$1592 \pm 401^*$	$-28 \pm 23$
4 weeks	$571 \pm 176^*$	$2282 \pm 1162^*$	$841 \pm 328^*$	$406 \pm 156^*$	$1255 \pm 283^*$	$-16 \pm 28$

\*  $P < 0.05$ .

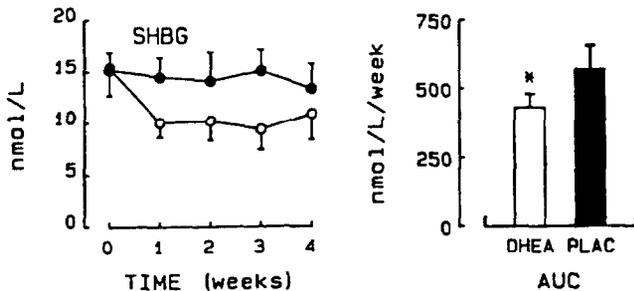


FIG. 2. Weekly serum SHBG levels (left) during oral administration of 1600 mg/day DHEA (O) or placebo (●) and integrated levels during the 4 weeks of administration (right). AUC, Area under the curve.

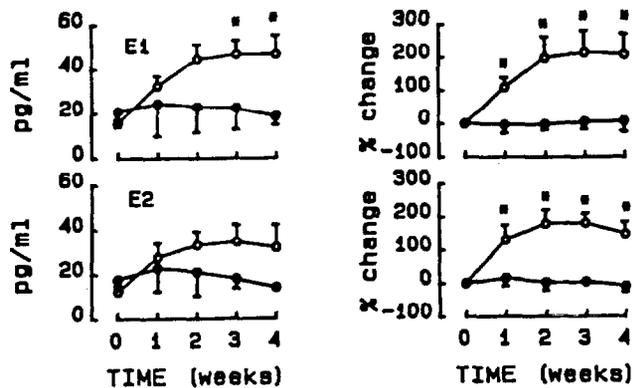


FIG. 3. Serum concentrations (left) and percent change (right) in  $E_1$  and  $E_2$  at weekly intervals during DHEA administration (O) or placebo (●). To convert metric units to System Internationale, multiply  $E_1 \times 3.699$  and  $E_2 \times 3.671$  to obtain picomoles per L.

LDL cholesterol, VLDL cholesterol, and total triglycerides showed a downward trend, but were not significantly lower during DHEA treatment compared with placebo treatment.

Weekly values for fasting serum glucose, insulin, and G6PD were similar throughout both DHEA and placebo treatments (Table 1). During oGTT, there was significant increase in insulin response after 4 weeks of DHEA treatment for both peak increments ( $1126 \pm 165$  vs.  $746 \pm 165$  pmol/L;  $P < 0.05$ ) and integrated values ( $P < 0.01$ ; Fig. 5). This was not accompanied by significant changes in glucose response. No differences were found in corresponding values during placebo treatment.

#### Anthropomorphic measures

Mean body weight did not change significantly during either DHEA or placebo treatment (Table 1). Percent body fat was also unaltered by DHEA treatment ( $47.3 \pm 0.9$  vs.  $47.3 \pm 0.7\%$ ) and was comparable to values at the beginning and end of the placebo treatment. Thus, no differences in anthropomorphic indices were detected in response to DHEA in our postmenopausal, relatively obese population. There were no significant differences

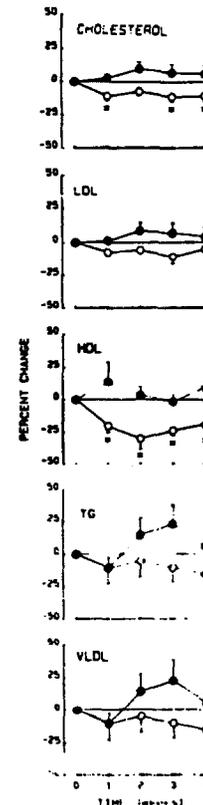


FIG. 4. Percent change in total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol, and total triglycerides at weekly intervals during DHEA administration at 1600 mg daily (O) or placebo (●). \*, Significant change from baseline. No significant changes were noted during placebo administration.

in dietary analysis based on 24-h recall between placebo and DHEA months.

#### Thyroid function

All subjects demonstrated a decline ( $P < 0.05$ ) in total  $T_4$  ( $111 \pm 15$  to  $76 \pm 15$  nmol/L) and an increase ( $P < 0.05$ ) in  $T_3RU$  ( $0.30 \pm 0.02$  to  $0.35 \pm 0.1$ ) during DHEA treatment, which were not seen with placebo. These changes were accompanied by a significant ( $P < 0.05$ ) decline in TBG levels ( $480 \pm 21$  to  $354 \pm 13$  nmol/L) in all subjects during DHEA treatment, while free  $T_4$  levels and total  $T_3$  and TSH levels were unchanged (Table 3).

#### Safety studies

Physical examination, liver function studies, complete blood counts, and urinalysis revealed no abnormalities or significant changes throughout the study. One subject reported increased facial hair during DHEA treatment, and one did so during placebo administration. No changes in sexual drive or appetite were reported. No other adverse consequences were noted.

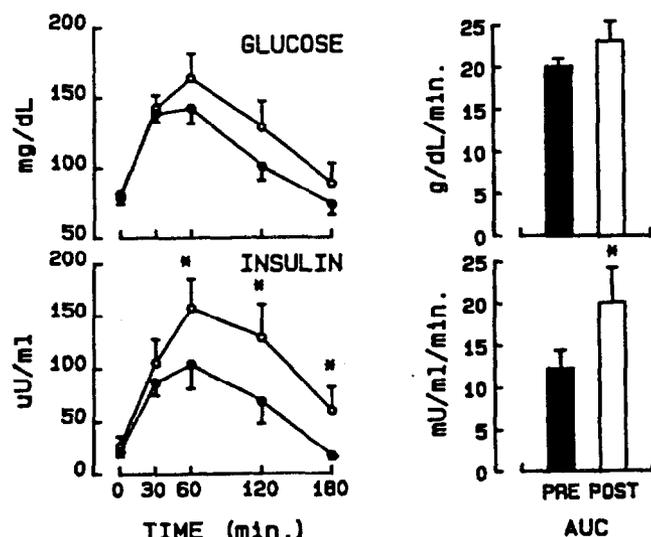


FIG. 5. Serum glucose and insulin responses to a 75-g oGTT before (●) and after (○) 28 days of DHEA at 1600 mg daily. \*,  $P < 0.05$ . Integrated serum glucose and insulin responses are on the right. Concentrations are in metric units. AUC, Area under the curve. To convert to System Internationale, multiply by the following factor: glucose  $\times 0.05551 = \text{mmol/L}$ , and insulin  $\times 7.715 = \text{pmol/L}$ .

### Discussion

Given the multiple beneficial effects of DHEA (and DS) demonstrated experimentally in animals and the lack of information concerning the biological action in humans, pharmacological assessments of DHEA should provide insights into the physiology of the age-related decline in both men and women. In a recent controlled study, DHEA administration (1600 mg/day, orally) for 28 days to a group of five young men (aged 22–25 yr) effected a reduced body fat mass, increased muscle mass, lower serum LDL cholesterol levels, and an unaffected tissue sensitivity to insulin (28). Based on these findings, the researchers inferred that the progressive decline in

DHEA and DS levels with aging may play a part in the increase in body fat and increased atherosclerosis with advancing age.

Our study in older postmenopausal women (aged 46–61) demonstrates that oral administration of pharmacological doses of DHEA results in a prompt and dramatic increase in serum levels of both  $\Delta^5$  and  $\Delta^4$  androgens. The parallel rise in serum levels of DHEA and DS between 60–180 min after DHEA administration suggests that rapid sulfation of circulating DHEA occurred and reflects an abundance of tissue steroid sulfatase in postmenopausal women. Increases in A and T were also observed within hours of DHEA administration, with increases in A being of similar magnitude to those in the  $\Delta^5$  androgens, implying extensive conversions of  $\Delta^5$  to  $\Delta^4$  androgens, the process of which requires  $3\beta$ -ol-hydroxysteroid dehydrogenase activity. This is in contrast to the low rate of peripheral conversion of  $\Delta^5$ - $\Delta^4$  steroids observed under physiological conditions (39). The increment in DHT (15-fold) was substantially greater than that in T (2.5-fold) in response to acute administration of DHEA. The reasons for this disproportionate large conversion of T to DHT are unclear. The availability of an abundance of  $5\alpha$ -reductase activity or a rapid induction by DHEA appear to be plausible explanations.

Sustained increases in serum androgen levels were noted throughout the 28-day course of the experiment. However, levels were invariably higher 2 weeks after the start of treatment and subsequently declined somewhat during weeks 3 and 4. Since serum levels obtained for DHEA itself were also highest after 2 weeks of treatment, these findings may be explained by a decrease in the amount of DHEA entering the circulation or a change in the metabolic clearance (MCR) of DHEA after prolonged oral administration, rather than by changes in enzyme activity within the androgen synthetic pathway. An increase in the MCR of DS has been demonstrated when

TABLE 3. Thyroid function studies before and after 28 days of treatment with DHEA and placebo in six postmenopausal women

	DHEA		Placebo	
	Day 0	Day 28	Day 0	Day 28
$T_4$ (nmol/L)	112 $\pm$ 15 (80–168)	76 $\pm$ 15* (53–100)	98 $\pm$ 9 (79–121)	114 $\pm$ 12 (80–150)
$T_3$ RU	0.30 $\pm$ .02 (.29–.34)	.35 $\pm$ .01* (.30–.38)	.31 $\pm$ .01 (.28–.31)	.30 $\pm$ .02 (.27–.36)
$T_3$ (nmol/L)	1.20 $\pm$ .14 (.89–1.80)	1.05 $\pm$ .10 (.78–1.35)	1.12 $\pm$ .10 (.94–1.49)	1.20 $\pm$ .12 (.86–1.64)
Free $T_4$ (pmol/L)	14.8 $\pm$ 1.03 (10.3–18.0)	15.1 $\pm$ 1.16 (10.3–18.0)	14.9 $\pm$ 1.03 (11.6–19.3)	14.8 $\pm$ 1.16 (11.6–19.3)
TBG (nmol/L)	480 $\pm$ 21 (404–530)	354 $\pm$ 13* (327–404)	428 $\pm$ 39 (387–489)	448 $\pm$ 43 (387–550)
TSH (mU/L)	3.7 $\pm$ 1.0 (1.7–8.5)	3.7 $\pm$ 0.5 (2.9–5.9)	3.6 $\pm$ 1.1 (1.9–6.2)	3.8 $\pm$ 1.2 (1.8–7.3)

The range is given in parentheses.

\*  $P < 0.05$ .

serum levels are raised acutely by constant infusion of DS, presumably as a result of a decreased fraction of the total circulating DS bound to albumin (40). Further experiments using alternate routes of DHEA administration will be required to elucidate the changes in first pass liver clearance after prolonged administration of pharmacological doses of DHEA.

In this experiment,  $E_1$  and  $E_2$  levels showed a pattern different from that of androgens. Unlike the dramatic and rapid increases in the latter, estrogen levels showed no acute increases, and their rise (2-fold) was much more gradual and sustained over the 4 weeks of treatment. This disparity between the conversion to potent androgen and estrogen after DHEA administration, especially in relatively obese postmenopausal women, is unexpected. A parallel decline in SHBG concentration, rendering progressively increased substrate availability for aromatization, cannot fully account for the disparity. An association between DHEA and SHBG levels has previously been noted (41) and was confirmed by our study.

A significant decline in total serum cholesterol and HDL cholesterol was seen during the first and subsequent weeks of DHEA administration. Although LDL cholesterol and other lipids were not significantly altered, they showed a downward trend. Consistent patterns of lipid profiles, as related to androgen influence, have not been found (42), but a decrease in HDL in high androgen environments has been observed (43). The lipid changes observed in this study may be a consequence of hepatic impact by pharmacological doses of DHEA. Studies using a transdermal delivery system in smaller doses of DHEA may disclose a different pattern of lipid profile. Despite the high circulating levels of potent androgens in our postmenopausal women, no increase in lean body mass was noted, and all anthropomorphic parameters were unaltered at the end of the 28-day treatment with either DHEA or placebo.

The results of our experiment in relatively obese postmenopausal women differ markedly from those observed in young normal weight men using identical doses and duration of DHEA administration (28), in which total cholesterol and LDL cholesterol levels declined during DHEA treatment. Although basal DS and A levels in our postmenopausal women were one third of those in the young males at the start of treatment, comparable levels were attained at the second week of treatment. Further, administration of the same dose of DHEA to young men resulted in no significant changes in serum testosterone level, whereas a 9-fold increase in testosterone and 20-fold increases in DHT were seen in our postmenopausal women. Thus, the relative increase in androgens as a result of DHEA administration in our population was far greater than that after administration of the same dose in younger men. The alterations in the HDL/LDL

ratio in our study is highly consistent with the impact of the high androgen environment (44). Our findings are in agreement with an earlier unblinded study using a single depot injection of 200 mg DHEA enanthrate to younger agonadal women, in which total cholesterol and HDL cholesterol were also shown to decline (29). Taken together, the different effects on lipid profiles in men and women suggest that although high levels of DHEA may be protective against cardiovascular morbidity in men (24), the same may not pertain in women.

The decrease in total  $T_4$  values in our study in the presence of normal free  $T_4$  values is readily explained by the reduction of TBG. This decrease in TBG is similarly reflected by an increased  $T_3RU$ , which is inversely related to TBG values in the presence of normal  $T_3$ . Therefore, all thyroid function alterations were accounted for by reduced levels of the single parameter, TBG. The lowering of both TBG and SHBG levels in response to pharmacological doses of DHEA administration and its subsequent conversion to potent androgens suggests inhibition of hepatic production of these binding proteins (45, 46).

Of particular interest was the finding of a 50% increase in both peak and integrated insulin responses to the oGTT in the face of an unaltered serum glucose response. This may be explained by the induction of an insulin-resistant state as the result of the profound increase in potent androgens during DHEA treatment (47). These data are consistent with the development of insulin resistance in male weightlifters ingesting large doses of androgenic steroids compared to matched weight lifters in the absence of exogenous steroids (48). These observations may further elucidate the mechanism by which insulin resistance occurs in polycystic ovary syndrome and is consonant with the hypothesis that increased androgens are at least in part responsible (49-53). Moreover, Shricok *et al.* (54) have shown that the insulin response to glucose is negatively correlated with serum DS, but positively correlated with serum T; further, a positive correlation between red blood cell insulin binding and serum DS, and a negative correlation with serum T were found. While an inverse relationship between insulin levels and serum DHEA and DS seems well established in both men (55) and women (56, 57), high levels of potent androgens may be sufficient to obviate this effect. This may explain the absence of altered glucose utilization in young men given DHEA (28) and the induction of insulin resistance in our postmenopausal women in whom potent androgen levels increased 20-fold during DHEA administration.

The clinical role of DHEA in postmenopausal steroid replacement regimens remains to be established. Although circulating levels of  $E_2$  and  $E_1$  achieved after 4 weeks of DHEA administration were lower than those of

estrogen supplements shown to be effective in preventing osteoporosis (58–60), the protective effects on bone density anticipated to result from the androgenic environment occasioned by DHEA may well be sufficient to compensate for the lower estrogen levels. At the very high androgen levels obtained in our experiment, adverse effects on HDL cholesterol and insulin sensitivity were seen that are not present in conventional menopausal estrogen replacement therapy (61–63). However, perenteral administration of lower doses of DHEA may result in more favorable lipid and carbohydrate metabolism alterations, consistent with the beneficial effects on atherogenesis and diabetes seen in animal studies (9–14). Moreover, further studies are required to examine the potential improvement in immunocompetence (15–23) and the decreased breast cancer rates (25) that may result from DHEA administration to postmenopausal women and thereby contribute to longevity.

Taken together, our results demonstrate that pharmacological doses of DHEA in postmenopausal women result in high circulating levels of all androgens along with a decline in SHBG and TBG and a modest increase in estrogens. A highly significant decline in both total cholesterol and HDL cholesterol was noted, while LDL cholesterol was unchanged. Moreover, insulin resistance was induced by the treatment. Further study using a different route and smaller doses of DHEA administration to circumvent the first pass hepatic effect is warranted.

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## Effects of Replacement Dose of Dehydroepiandrosterone in Men and Women of Advancing Age\*

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### ABSTRACT

Aging in humans is accompanied by a progressive decline in the secretion of the adrenal androgens dehydroepiandrosterone (DHEA) and DHEA sulfate (DS), paralleling that of the GH-insulin-like growth factor-I (GH-IGF-I) axis. Although the functional relationship of the decline of the GH-IGF-I system and catabolism is recognized, the biological role of DHEA in human aging remains undefined. To test the hypothesis that the decline in DHEA may contribute to the shift from anabolism to catabolism associated with aging, we studied the effect of a replacement dose of DHEA in 13 men and 17 women, 40–70 yr of age. A randomized placebo-controlled cross-over trial of nightly oral DHEA administration (50 mg) of 6-month duration was conducted. During each treatment period, concentrations of androgens, lipids, apolipoproteins, IGF-I, IGF-binding protein-1 (IGFBP-1), IGFBP-3, insulin sensitivity, percent body fat, libido, and sense of well-being were measured. A subgroup of men (n = 8) and women (n = 5) underwent 24-h sampling at 20-min intervals for GH determinations.

DHEA and DS serum levels were restored to those found in young adults within 2 weeks of DHEA replacement and were sustained throughout the 3 months of the study. A 2-fold increase in serum levels

of androgens (androstenedione, testosterone, and dihydrotestosterone) was observed in women, with only a small rise in androstenedione in men. There was no change in circulating levels of sex hormone-binding globulin, estrone, or estradiol in either gender. High density lipoprotein levels declined slightly in women, with no other lipid changes noted for either gender. Insulin sensitivity and percent body fat were unaltered. Although mean 24-h GH and IGFBP-3 levels were unchanged, serum IGF-I levels increased significantly, and IGFBP-1 decreased significantly for both genders, suggesting an increased bioavailability of IGF-I to target tissues. This was associated with a remarkable increase in perceived physical and psychological well-being for both men (67%) and women (84%) and no change in libido.

In conclusion, restoring DHEA and DS to young adult levels in men and women of advancing age induced an increase in the bioavailability of IGF-I, as reflected by an increase in IGF-I and a decrease in IGFBP-1 levels. These observations together with improvement of physical and psychological well-being in both genders and the absence of side-effects constitute the first demonstration of novel effects of DHEA replacement in age-advanced men and women. (*J Clin Endocrinol Metab* 78: 1360–1367, 1994)

**A**GING in man is associated with reduced protein synthesis, decreased lean body mass and bone mass, and increased body fat (1). These body composition changes are accompanied by a progressive decline of adrenal secretion of dehydroepiandrosterone (DHEA) and its sulfate ester (DS) (2) paralleling that of the GH-insulin-like growth factor-I (GH-IGF-I) system (1). Although the GH-IGF-I system is recognized as a trophic factor in promoting cellular growth and metabolism at multiple sites (1, 3), the biological role of DHEA and DS in humans remains elusive. Based on animal experiments, DHEA may be viewed as a multifunctional steroid with protective roles in many aspects of cellular well-being, especially aging-associated deficits (4–7). The rele-

vance of these findings to human biology and diseases is perplexing, because adrenal production of DHEA or DS in these experimental animals is either minute or does not exist; humans and nonhuman primates are the only species with the capacity to synthesize and secrete these adrenal androgens in quantities surpassing all other known steroids (8–10).

Epidemiological data support beneficial effects of DHEA and DS. Low serum DS levels are correlated with increased cardiovascular morbidity in men (11), breast cancer in women (12), and the decline of immunocompetence during aging (13). However, prospective studies (14, 15) failed to confirm earlier reports of an inverse association of plasma DS levels and angiographically defined coronary atherosclerosis in men (16) and a positive correlation of serum DS levels and bone mineral density in aging women (17).

In healthy young men, using suprapharmacological doses of DHEA (1600 mg/day) for 4 weeks, Nestler *et al.* (18) reported a decrease in cholesterol and low density lipoprotein (LDL) levels as well as a 31% decrease in body fat without weight changes, implying an increase in muscle mass (18); these findings, however, were not confirmed by subsequent reports using the same dose of DHEA in obese (19) and nonobese (20) young men and in postmenopausal women (21). All clinical studies thus far were conducted with mega-

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uses of DHEA, which may induce responses beyond its physiological action or even induce down-regulation of cellular response.

In the present study, we tested the supposition that restoring extracellular levels of DHEA and DS in individuals of advancing age to levels in young adults may have beneficial effects on neuroendocrine-metabolic functions. We report here the results of a replacement dose of 50 mg DHEA administered orally at bedtime to 17 women and 13 men, aged 40–70 yr, in a double blind, placebo cross-over trial of 6-month duration.

## Materials and Methods

### Subjects

Subjects were recruited by advertisement. 17 women (mean, 54.5 ± 1.9 yr) and 13 men (mean, 53.7 ± 2.5 yr), aged 40–70 yr, were entered into the study. There were 5 subjects of each gender in each decade of the age group, except for only 3 men in the fourth decade and 6 women each in the fifth and sixth decades. All subjects were nonobese with a body mass index (BMI) of 20–28 kg/m<sup>2</sup> (mean, 24.5 ± 0.6 kg/m<sup>2</sup> for women, 26.6 ± 0.7 kg/m<sup>2</sup> for men) and were nonsmokers; they were taking no medications and had stable dietary and exercise regimens. Of the 17 women, 2 were premenopausal, and 15 were menopausal. Of these 15, 8 were receiving menopausal estrogen replacement (previous hysterectomy), whereas the other 7 women were receiving no menopausal replacement therapy. Medical illness was excluded by history, physical examination, blood chemistry profile (including renal, hepatic, and thyroid panels), urinalysis, and complete blood count. All subjects completed the protocol. The protocol was approved by the Committee on Investigations Involving Human Subjects of the University of California-San Diego. All subjects gave oral and written informed consent.

### Study design

The study design was a randomized, double blind, placebo cross-over trial of 6-month duration. Sample size in this trial was calculated using a computer software package (Power and Sample Size by J. L. Hintze) by estimating the  $\alpha$  error at 0.05, the  $\beta$  error at 0.25 (power 75%), and  $\Delta$  (size of treatment effect sought) of 1 so of the measurement of the outcome variable.

The daily replacement oral dose of 50 mg DHEA was determined by taking into account the MCR of 11–15 L/day, a daily production rate of 18–28 mg for DS, interconversion rates of DHEA to DS of 7.7% and DS to DHEA of 30% (22, 23), estimated endogenous serum levels in individuals of advancing age of 20–40% young adult levels (2, 24), and absorption of 50% of an oral dose (21).

Each subject received 3 months of DHEA and 3 months of placebo at bedtime in random order. Compliance was checked by pill counts and monthly refills and retrospectively by DHEA and DS levels. All subjects were instructed by a nutritionist to continue their current and usual dietary and exercise regimens. A questionnaire was administered at monthly intervals for dietary recall, using household measures (25). These records were coded and analyzed using the Nutrition III version 7 software program. No major changes in the diet/exercise regimen of individuals occurred over the duration of the trial.

### Study protocol and procedures

Potential adverse effects were monitored by means of interviews, physical examinations, and standard laboratory tests. Subjects were seen monthly as well as 2 weeks after their baseline and 3 and 6 month visits. Blood was drawn at each visit between 0800–0900 h after an overnight fast for determination of serum steroid hormones and sex hormone-binding globulin (SHBG) levels as well as liver function, renal function, electrolytes, complete blood count, and urinalysis.

At the baseline, 3 month, and 6 month visits, an additional fasting

blood sample was collected for lipids, IGF-I, IGF-binding protein-1 (IGFBP-1), IGFBP-3, insulin, and glucose determinations. During each of these visits, the heights and weights of all subjects were recorded as well as an estimation of percent body fat by bioelectric impedance (B-101A RJL System, Detroit, MI) (26–28). Insulin sensitivity was assessed in all subjects by the modified, minimally sampled, iv glucose tolerance test (29): administration of 300 mg/kg dextrose as an iv bolus over 1 min, followed 20 min later by an iv bolus of regular insulin (0.03 U/kg). Frequent blood samples were obtained (at 0, 2, 4, 8, 19, 22, 30, 40, 50, 70, 90, and 180 min) for determinations of serum glucose and insulin. Insulin sensitivity and glucose effectiveness were analyzed using the MINMOD computer program (30). In a subgroup of five subjects, insulin sensitivity was further measured using the hyperinsulinemic euglycemic clamp method at baseline and 3 and 6 months. A visual analog scale of libido and an open ended questionnaire for self-assessment of well-being and activities as well as side-effects were administered at the 3 (cross-over) and 6 month visits.

A subgroup of 13 of the 30 subjects (8 men and 5 women) was admitted to the Clinical Research Center for 24-h frequent blood sampling (every 20 minutes) from 0800–0800 h for determination of serum GH profiles at baseline and 3 and 6 months. Meals were served at 0800, 1200, and 1700 h, and subjects slept from 2200–0700 h.

Another subgroup of five subjects (three men and two women) was admitted to the Clinical Research Center for hyperinsulinemic euglycemic clamp studies, according to previously described procedures (31), to assess tissue sensitivity to insulin, expressed as the glucose disposal rate (milligrams per kg/min) at baseline and 3 and 6 months. Briefly, a catheter was inserted into an antecubital vein for infusion of insulin and glucose. A second catheter was inserted retrogradely into a dorsal hand vein, which was kept in a heating device, for sampling of arterialized venous blood. Recombinant human regular insulin was infused via a Harvard pump at a rate of 40 mU/m<sup>2</sup>·min for a total of 240 min. Serum glucose was maintained at 4.7–5.3 mmol/L by adjustments of a variable infusion of 20% glucose based on the glucose values determined at 5-min intervals. Whole body glucose disposal rates were calculated by determining the mean of data from the last 60 min of the study on each occasion, corrected for residual hepatic glucose output measured by isotope dilution (32).

### Assays

All hormone measurements for an individual subject were performed in the same assay. Serum concentrations of DHEA, DS, testosterone (T), androstenedione (A'dione), estrone (E<sub>1</sub>), estradiol (E<sub>2</sub>), and dihydrotestosterone (DHT) were measured by specific RIAs, previously described (33). The sensitivity of the assays were 0.5 nmol/L for DHEA, 0.6  $\mu$ mol/L for DS, 0.3 nmol/L for T, 0.6 nmol/L for A'dione, 42 pmol/L for E<sub>1</sub>, 44 pmol/L for E<sub>2</sub>, and 0.2 nmol/L for DHT. The intra- and interassay coefficients of variation were, respectively, 8% and 9% for DHEA, 5% and 7% for DS, 5% and 6% for T, 4% and 11% for A'dione, 11% and 12% for E<sub>1</sub>, 9% and 10% for E<sub>2</sub>, and 8% and 11% for DHT.

Serum GH levels were measured by an established RIA (34) with a sensitivity of 0.5  $\mu$ g/L and intra- and interassay coefficients of 5% and 9%, respectively. IGF-I, IGFBP-1, and IGFBP-3 were determined by established RIAs at Nichols Institute Reference Laboratories (San Juan Capistrano, CA). IGF-I was measured after acid-ethanol extraction (35, 36) with an assay sensitivity of 0.1 ng/mL and intra- and interassay coefficients of variation of 5.0% and 10.2%, respectively. RIAs for IGFBP-1 and IGFBP-3 have sensitivities, respectively, of 1 ng/mL and 0.1 mg/L and intra- and interassay coefficients of variation, respectively, of less than 5% and 14.2% for IGFBP-1 and 3% and 7.2% for IGFBP-3 (37, 38).

SHBG was measured by time-resolved fluoroimmunoassay (Delfia SHBG kit, Wallac, Gaithersburg, MD), with a sensitivity of 0.8 nmol/L and intra- and interassay coefficients of variation, respectively, of 7% and 9%. Serum glucose concentrations were determined by the glucose oxidase method (Yellow Springs Instrument Co. analyzer, Yellow Springs, OH). Serum insulin levels were measured by a double antibody RIA, with an assay sensitivity of 2.1  $\mu$ U/mL, and intra- and interassay coefficients of variation of 7% and 9%, respectively (39).

Serum total cholesterol and triglycerides were measured by previously described enzymatic methods (40, 41). Very low density lipoprotein was

calculated by dividing the triglyceride value by 5. LDL cholesterol was calculated using the formula: LDL = cholesterol - (HDL + very low density lipoprotein). HDL cholesterol was enzymatically determined after sodium phosphotungstate/magnesium precipitation of other lipids. Apolipoprotein-A-1 and -B were quantified by Behring nephelometry. Coefficients of variations vary from 2-10%. Complete blood counts, liver function studies, thyroid panel, electrolytes, and urinalysis, used to monitor subject safety, were performed in a commercial laboratory (MetWest Unilabs, San Diego, CA).

#### Analyses of data

GH pulsatile activity was analyzed using the Cluster pulse detection algorithm, with a cluster configuration of  $2 \times 2$  and  $t$  statistics of  $2.5 \times 2.5$  (42). Differences between all steroid hormone concentrations as well as SHBG at the monthly time points were performed by two-way analysis of variance with repeated measures, with factors gender and treatment, followed by Dunnett's testing. Lipids, BMI, percent body fat, insulin sensitivity, glucose effectiveness, glucose disposal rates, GH parameters, IGF-I, IGFBP-1, and IGFBP-3 were analyzed by an analogous two-way analysis of variance, followed by *post-hoc* Dunnett's testing for comparing treatment groups (DHEA and placebo) to baseline (control). Differences in self-reported well-being and libido were analyzed by paired  $t$  tests (two tailed). Data are presented as the mean ( $\pm$  SE), and  $P < 0.05$  was considered significant for all analyses.

### Results

#### Steroid hormone changes

In response to DHEA administration, within 2 weeks, serum levels of DHEA and DS were elevated from placebo values in both men ( $8.47 \pm 0.8$  to  $14.72 \pm 1.4$  nmol/L;  $3.5 \pm 0.3$  to  $10.1 \pm 1.2$   $\mu$ mol/L;  $P < 0.001$ ) and women ( $7.19 \pm 0.5$  to  $16.13 \pm 1.3$  nmol/L;  $1.78 \pm 0.17$  to  $9.27 \pm 0.76$   $\mu$ mol/L;  $P < 0.001$ ) respectively (Fig. 1). The incremental levels attained were in the range of young adult levels (2). These levels were maintained throughout the 3-month trial based on monthly determinations and were normalized within 2 weeks after discontinuation of DHEA (data not shown). Serum levels of DS attained with DHEA treatment were not significantly correlated to baseline DS levels for men ( $r = 0.21$ ;  $P = 0.5$ ) or women ( $r = -0.22$ ;  $P = 0.4$ ).

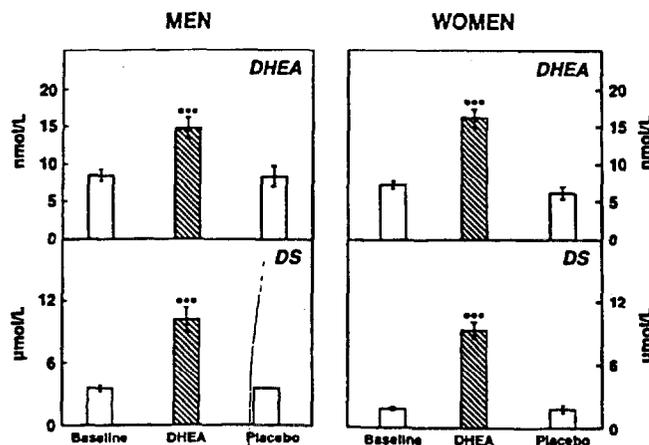


FIG. 1. Mean ( $\pm$ SE) serum DHEA and DS concentrations in men (left panel) and women at baseline, after 12 weeks of oral administration of 50 mg DHEA nightly, and after 12 weeks of placebo administration. \*\*\*,  $P < 0.005$  compared with placebo values.

Biotransformation of DHEA to potent androgens occurred selectively in women (Fig. 2, right panel). Between baseline and 12 weeks of DHEA administration in women, serum A'dione increased from  $1.33 \pm 0.13$  to  $3.0 \pm 0.19$  nmol/L ( $P < 0.001$ ), serum T from  $0.72 \pm 0.07$  to  $1.46 \pm 0.14$  nmol/L ( $P < 0.001$ ), and serum DHT from  $0.32 \pm 0.03$  to  $0.9 \pm 0.1$  nmol/L ( $P < 0.001$ ), whereas serum SHBG concentrations exhibited a tendency to decline ( $105.5 \pm 12.3$  to  $81.2 \pm 10.6$  nmol/L) that was not statistically significant. In men, there was no significant change in serum T, DHT, or SHBG concentrations with baseline, DHEA, or placebo administration. Serum A'dione levels, however, increased significantly during DHEA treatment ( $1.86 \pm 0.11$  to  $2.23 \pm 0.14$  nmol/L;  $P < 0.01$ ; Fig. 2, left panel). Serum levels of  $E_1$  and  $E_2$  were not significantly altered, in men ( $E_1$ ,  $89.4 \pm 5.9$  vs.  $95.0 \pm 7.8$  pmol/L;  $E_2$ ,  $87.7 \pm 4.8$  vs.  $78.3 \pm 6.6$  pmol/L), and women ( $E_1$ ,  $256.2 \pm 58.5$  vs.  $268.2 \pm 58.8$  pmol/L;  $E_2$ ,  $144.7 \pm 35.2$  vs.  $107.3 \pm 20.6$  pmol/L).

#### Lipids (Table 1)

Serum lipids, triglycerides, and apolipoprotein-B and -A-1 did not change in men and women, with the exception of serum HDL in women, which was reduced during DHEA

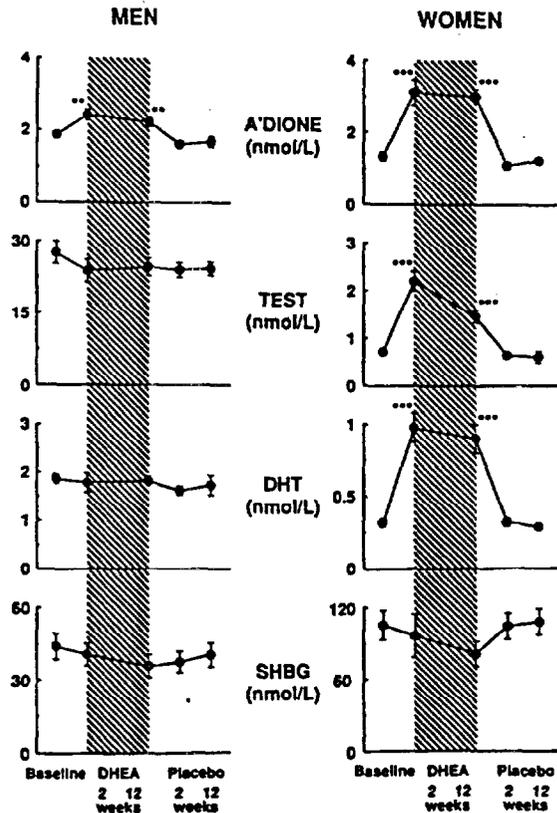


FIG. 2. Serum concentrations of androgens and SHBG in men (left panel) and women at baseline, 2 and 12 weeks after oral administration of 50 mg DHEA nightly, and 2 and 12 weeks after placebo administration. The shaded area represents the DHEA treatment period. \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.005$  (compared with basal values). TEST, T.

**TABLE 1.** Effects of replacement dose of DHEA and placebo on cholesterol and lipoprotein levels in men (n = 13) and women (n = 17)

	Men	Women
<b>Cholesterol (mg/dL)</b>		
Placebo	204.3 ± 7.9	223.7 ± 5.8
DHEA	209.9 ± 9.4	218.3 ± 6.9
<b>HDL (mg/dL)</b>		
Placebo	45.4 ± 2.5	68.7 ± 2.6
DHEA	44.8 ± 1.7	63.4 ± 2.7*
<b>LDL (mg/dL)</b>		
Placebo	140.4 ± 6.9	141.2 ± 6.2
DHEA	147.5 ± 8.5	136.0 ± 4.9
<b>Apo-A1 (mg/dL)</b>		
Placebo	165.4 ± 5.3	222.8 ± 8.1
DHEA	167.4 ± 6.1	213.7 ± 8.1
<b>Apo-B (mg/dL)</b>		
Placebo	143.0 ± 7.3	137.2 ± 5.8
DHEA	149.8 ± 8.5	137.4 ± 5.9
<b>TG</b>		
Placebo	114.5 ± 13.5	102.5 ± 9.2
DHEA	107.7 ± 10.5	94.2 ± 7.4

HDL, High density lipoprotein; Apo-A1, apolipoprotein-A1; Apo-B, apolipoprotein-B; TG, triglycerides. Values are the mean ± SEM.

\*  $P < 0.05$  compared to placebo.

treatment (68.7 ± 2.6 to 63.4 ± 2.7 mg/dL;  $P < 0.05$ ) compared with placebo.

#### Anthropometric measures and glucose metabolism (Table 2)

There was no significant change in anthropometric indices (percent body fat or BMI) during either DHEA or placebo treatment in men or women.

Insulin sensitivity and the insulin-independent fractional glucose disappearance, glucose effectiveness, as determined by Bergman's modified minimal model technique, did not change significantly during DHEA administration in either men or women. Similarly, glucose disposal rates as determined by the hyperinsulinemic euglycemic clamp in three men and two women showed no significant difference with DHEA or placebo administration (DHEA, 7.64 ± 0.56 mg/kg·min; placebo, 8.41 ± 1.26 mg/kg·min).

#### Well-being and libido

An improved sense of well-being was self-reported by the majority of women (82%) as well as men (67%) after 12 weeks of DHEA administration, whereas less than 10% reported any change after placebo administration (Fig. 3, left panel). Specific statements of well-being ranged from improved quality of sleep, more relaxed, increased energy to

better ability to handle stress. No difference was noted in libido while subjects were receiving DHEA compared to the placebo group (Fig. 3, right panel). Of note, there were five subjects who self-reported marked improvements of preexisting joint pains and mobility during DHEA replacement.

#### GH-IGF axis

There was no difference noted in GH pulse number per 24 h, mean amplitude, or transverse mean in the subset of 13 subjects (8 men and 5 women) who underwent 24-h frequent blood sampling for GH (Table 3). A representative example of 24-h GH pulsatile activity at baseline and in response to the administration of DHEA vs. placebo is displayed in Fig. 4.

In response to DHEA administration, men (Fig. 5, left panel) showed an elevation of serum IGF-I levels (151.3 ± 10.2 to 180.1 ± 15.4 ng/mL;  $P < 0.01$ ) and a decline in IGFBP-1 levels (28.7 ± 3.3 to 20.4 ± 3.5 ng/mL;  $P < 0.05$ ), resulting in an increased IGF-I/IGFBP-1 ratio (8.3 ± 1.6 to 12.7 ± 2.9;  $P < 0.05$ ). A similar finding was seen in women (Fig. 5, right panel), with elevation of serum IGF-I levels (140.8 ± 14.0 to 157.4 ± 16.4 ng/mL;  $P < 0.05$ ) and a decline in IGFBP-1 levels (53.2 ± 6.6 to 41.3 ± 5.7 ng/mL;  $P < 0.01$ ), resulting in an increased IGF-I/IGFBP-1 ratio (4.2 ± 1.1 to 6.6 ± 1.9;  $P < 0.05$ ). There was no change in IGFBP-3 levels in men (2.8 ± 0.2 to 2.6 ± 0.2 ng/mL) or women (2.8 ± 0.1 to 2.8 ± 0.1 mg/L; Fig. 5).

Although the baseline IGF-I levels were similar, a significant gender difference was noted in baseline IGFBP-1 levels, with women having a 2-fold higher level than men (women, 51.0 ± 7.0; men, 25.5 ± 4.1;  $P < 0.01$ ), which confirms the report of Yeoh and Baxter (43). Consequently, a significantly lower IGF-I/IGFBP-1 ratio occurs in women ( $P < 0.05$ ; Fig. 6A). Given this gender difference, the data were further analyzed to determine whether the relative changes from placebo group values for men and women differed. As shown in Fig. 6B, the individual percent change from placebo for IGF-I and IGFBP-1 values in both genders were fairly uniform. An increase in IGF-I in men (11.3 ± 4.8%;  $P < 0.05$ ) and women (9.7 ± 5.0%;  $P < 0.05$ ) as well as a significant decrease in IGFBP-1 in men (20.7 ± 6.7%;  $P < 0.05$ ) and women (18.3 ± 6.2%;  $P < 0.05$ ) imposed by DHEA administration relative to placebo group values were evident. Consequently, the IGF-I/IGFBP-1 ratio, an index that may reflect an overall increase in the bioavailability of IGF-I, was increased in both men (52.0 ± 15.9%;  $P < 0.05$ ) and women (54.1 ± 15.1%;  $P < 0.05$ ) to a similar extent. Whether

**TABLE 2.** Effects of replacement dose of DHEA and placebo on body composition and glucose metabolism in men (n = 13) and women (n = 17)

	Men		Women	
	Placebo	DHEA	Placebo	DHEA
% Fat	22.1 ± 1.2	21.9 ± 1.3	31.0 ± 1.0	31.3 ± 1.0
BMI (kg/m <sup>2</sup> )	27.3 ± 0.8	27.0 ± 0.7	24.8 ± 0.7	24.7 ± 0.7
Insulin sensitivity (×10 <sup>-4</sup> min/μU·mL)	2.89 ± 0.6	3.69 ± 0.5	3.72 ± 0.5	2.90 ± 0.4
Glucose effectiveness (min <sup>-1</sup> )	0.022 ± .002	0.019 ± 0.001	0.018 ± 0.001	0.024 ± .002

Values are the mean ± SEM.

FIG. 3. Percentage of men and women who self-reported an improved sense of well-being after 12 weeks of oral administration of 50 mg DHEA nightly and after 12 weeks of placebo administration (left panel). \*\*\*,  $P < 0.005$  compared with placebo values. Scored values of libido on a visual analog scale in men and women after 12 weeks of oral administration of 50 mg DHEA nightly and after 12 weeks of placebo administration are shown. \*\*,  $P < 0.01$  compared with opposite gender values.

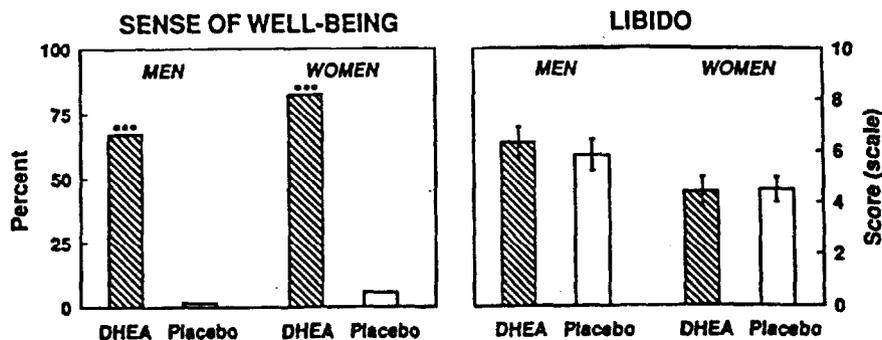


TABLE 3. Effects of replacement dose of DHEA and placebo on 24-h GH characteristics in men (n = 8) and women (n = 5)

	Pulse no./24 h	Amplitude (µg/L)	Transverse mean (µg/L)
Baseline	6.00 ± 0.45	3.65 ± 0.73	2.19 ± 0.27
DHEA	5.23 ± 0.55	2.49 ± 0.45	2.05 ± 0.28
Placebo	5.54 ± 0.50	3.17 ± 0.76	2.05 ± 0.27

Values are the mean ± SEM.

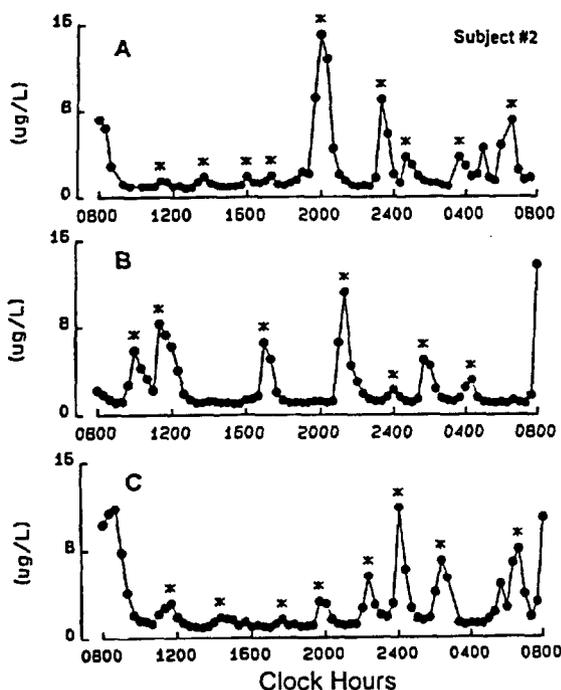


FIG. 4. Twenty-four-hour serum GH pulsatile pattern in a representative subject (a 47-yr-old woman, subject 2) at baseline (A), after 12 weeks of oral administration of 50 mg DHEA nightly (B), and after 12 weeks of placebo (C) administration. \*, Identified pulses.

postmenopausal women were with (n = 8) or without (n = 7) estrogen replacement was not a significant factor in the relative increase in the ratio of IGF-I/IGFBP-1 (with estrogen, 48%; without estrogen, 66%;  $P = NS$ ). Serum levels of IGF-I and IGFBP-1 attained with DHEA treatment were not significantly correlated to basal levels.

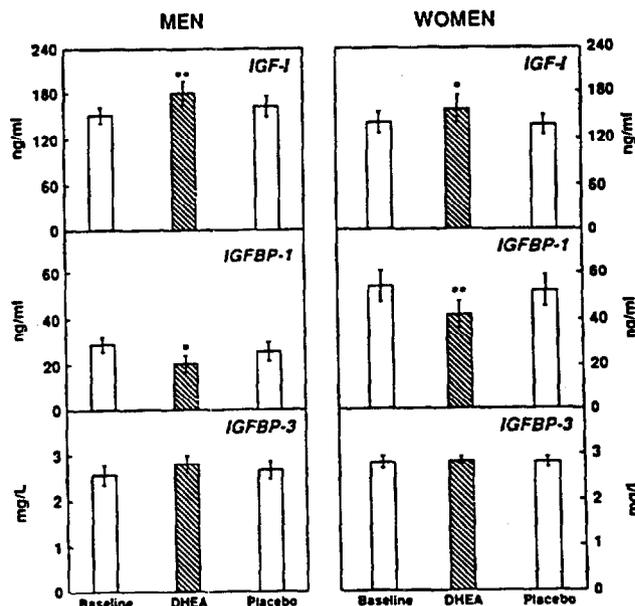


FIG. 5. Serum IGF-I, IGFBP-1, and IGFBP-3 concentrations in men (left panel) and women at baseline, after oral administration of 50 mg DHEA nightly for 12 weeks, and after placebo for 12 weeks. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$  (compared with placebo values).

Safety studies

Physical examination, hepatic and thyroid studies, complete blood count, and urinalysis revealed no abnormalities or significant changes throughout the study. One female subject reported increased facial hair during DHEA treatment, and one did so during placebo; both resolved by the end of 3 months of the other treatment period.

Discussion

In the present study, we have demonstrated that a calculated replacement dose of 50 mg DHEA administered orally at bedtime to men and women of advancing age restored DHEA and DS to levels seen in the second decade of life (2). These levels were sustained throughout the 3 months of DHEA administration. The use of physiological replacement rather than pharmacological doses of DHEA may be of critical importance in determining the effects of DHEA and DS in the aging population, because of its ability to undergo

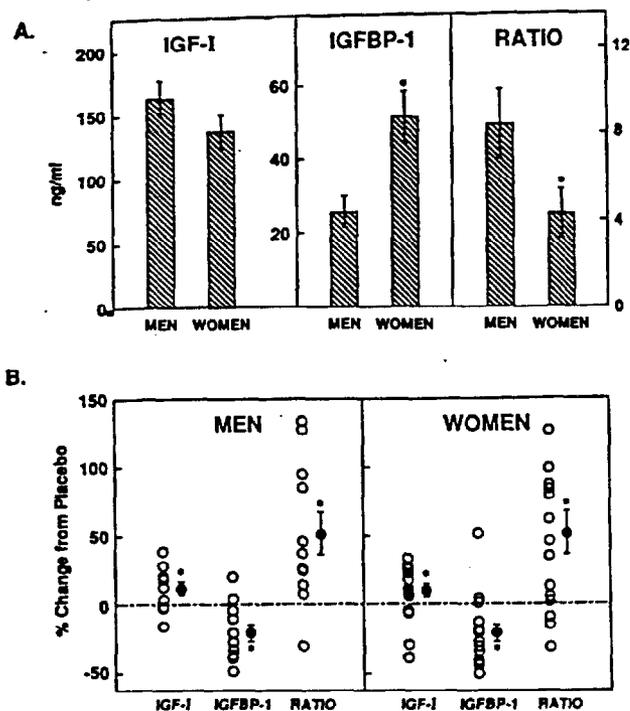


FIG. 6. A, Baseline (mean  $\pm$  SE) serum IGF-I and IGFBP-1 concentrations and their ratio (IGF-I/IGFBP-1) in men and women. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$  (compared with opposite gender value). B, Percent change from placebo of individual values for serum IGF-I, IGFBP-1, and IGF-I/IGFBP-1 ratio after 12 weeks of oral administration of 50 mg DHEA nightly in men and women. The mean  $\pm$  SE of each measurement are also shown. \*,  $P < 0.05$  compared with placebo.

rapid biotransformation to potent androgens, which, in turn, may have a biological impact on target tissue, including anabolic effects. To date, only one study was designed to address the issue of the pharmacological impact of DHEA. Mortola and Yen (21), using a 1600-mg daily oral dose of DHEA in postmenopausal women (aged 46–61 yr) for 4 weeks, demonstrated a marked biotransformation of DHEA to potent androgens and estrogens. The increments reached 9-fold for T, 20-fold for A'dione and DHT, and 2-fold for E<sub>1</sub> and E<sub>2</sub>. This hyperandrogenic state imposed by a pharmacological dose of DHEA was associated with a significant decline in SHBG, T<sub>4</sub>-binding globulin, total cholesterol, and high density lipoprotein cholesterol and the appearance of insulin resistance. These marked endocrine-metabolic changes were not observed in our present study with a replacement dose of DHEA. The increments in androgens, but not estrogens, were minimal and occurred selectively in women. Most importantly, these levels were within the normal ranges of adult women (44). Thus, within our experimental design, the results obtained may reflect the physiological actions of DHEA *in vivo*.

The most intriguing observation made in the present study was changes in the IGF-I system. DHEA replacement induced an approximately 10% rise in serum IGF-I levels and an approximately 19% decline in IGFBP-1 levels, resulting in an elevation of IGF-I/IGFBP-1 ratio by 50% in both men

and women. These changes occurred independent of gender differences in baseline IGFBP-1 levels and were unaccompanied by changes in insulin sensitivity and 24-h GH or GH-dependent IGFBP-3 levels.

IGF-I, a potent metabolic growth factor, is regulated by GH and secreted constitutively by the liver without storage (45). To maintain steady levels in the circulation, IGF-I is bound to a series of IGFBPs. The major BP in serum is IGFBP-3, which possesses the highest binding affinity for IGFs and is completely saturated (46). Serum IGFBP-1 is present with a relatively low binding affinity and at a 100-fold lower concentration than IGFBP-3, but is unsaturated and binds IGFs readily (45–47). That IGFBP-1 may play a physiological role by modulating the availability of IGF-I for metabolic homeostasis is suggested by its ability *in vivo* to block IGF-I action and increase blood glucose levels after the administration of IGFBP-1 (45). IGFBP-1 is mainly expressed in the liver, and its release is suppressed by insulin (45–47) and GH (48). Given this background, the effect of DHEA on IGF-I/IGFBP-1 observed in the present study appears to be operating outside the known regulatory mechanism of the GH-IGF-I and insulin-IGFBP-1 axes. In the absence of a known mechanism of DHEA action, we speculate that restoration of DHEA levels in men and women of advancing age may exert a stimulatory action on either the hepatic production of IGF-I or the generation of GH receptors, thereby enhancing the effectiveness of ambient GH levels for IGF-I production. In the same context, hepatic production of IGFBP-1 may be inhibited by DHEA to account for the increased IGF-I/IGFBP-1 ratio without a discernible change in GH or insulin levels. The possibility that DHEA may represent a previously unrecognized physiological regulator of the IGF-I/IGFBP-1 system should be considered an issue that deserves further investigation.

It is also not known whether this relative increase in the bioavailability of IGF-I in response to DHEA in time may exert a metabolic impact on anabolism or attenuate ongoing catabolism. The role of IGF-I in reversing catabolism has been recognized in experiments of GH administration in aging populations (3). Several IGF-I infusion studies, as recently reviewed by Clemmons (49), have shown that acute elevation of IGF-I levels can induce a glucose-lowering and protein-sparing effect in healthy adult men, and this anabolic action of IGF-I is more evident in nutritionally deprived individuals. The question of long term target tissue exposure to endogenously generated IGF-I bioavailability in response to restoration of DHEA levels was not addressed in the present experiment. Nevertheless, our findings represent the first demonstration of a biological effect of DHEA in men and women of advancing age. Further studies at selected target tissue responses are warranted.

Although we are fully cognizant of the limitations of self-reported mood-behavioral changes in response to an experimental agent, the self-reported increase in well-being by the large majority of our subjects during DHEA treatment should be viewed as a reliable outcome in this double blind, placebo-controlled study. Self-reported changes include increased energy, deeper sleep, improved mood, more relaxed feeling,

and better ability to handle stressful events. The lack of corresponding changes in libido support the validity of the positive event of improved physical and psychological capacity during DHEA, but not placebo, treatment.

The mechanism underlying the array of individual well-being enhancements in response to replacement doses of DHEA in both men and women is unclear. It is unrelated to the small increments in potent androgens, as this occurred only in women. The possibility of a central effect of DHEA should be considered. Substantial experimental evidence now exists showing that DHEA and DS bind to the  $\gamma$ -aminobutyric acid<sub>A</sub>-receptor complex and function as an antagonist or negative modulator, resulting in activation of neuronal excitability *in vitro* (see Ref. 50 for review). Further, a memory-enhancing effect and inhibition of aggressive behavior have been demonstrated *in vivo* (50). DHEA and DS have been found in the human brain and may mediate a distinct allosteric mode of interaction with the  $\gamma$ -aminobutyric acid<sub>A</sub> receptor, as in rodents (50). However, it is not known whether incremental changes in circulating DHEA can be delivered to brain target sites. Alternatively, the potential improvement of cellular well-being by trophic effects of IGF-I may manifest as improved capacity of physical and psychological performances. To clarify these postulates would require a multidisciplinary approach.

In conclusion, our results support the hypothesis that DHEA may indeed have a biological function in man, and that restoration of DHEA and DS levels in age-advanced men and women with a replacement dose of DHEA induced an increase in bioavailable IGF-I, which, with time, may result in an improvement in catabolic processes and physical/psychological well-being. Future trials are warranted to identify the biological end points of elevated bioavailable IGF-I in healthy aging individuals.

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## Replacement of DHEA in Aging Men and Women

### Potential Remedial Effects<sup>a</sup>

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Aging in men is associated with reduced protein synthesis, decreased lean body mass and bone mass, and increased body fat.<sup>1</sup> These body composition changes are accompanied by a progressive decline in adrenal secretion of dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS),<sup>2</sup> paralleling those of the growth hormone (GH)-insulin-like growth factor-1 (GH-IGF-1) system and immune function.<sup>1,3</sup> Although the GH-IGF-1 system is recognized to promote cellular growth and metabolism at multiple sites<sup>1,4</sup> and to modulate the immune system in health and disease,<sup>3,5-7</sup> the biologic function of DHEA and DHEAS in humans remains elusive. Extensive animal experiments have shown that DHEA may have immunoenhancing<sup>8-11</sup> and protective effects against viral infection,<sup>12</sup> glucocorticoid-induced thymic involution,<sup>13</sup> autoantibody formation,<sup>14</sup> and age-related deficits such as obesity, cardiovascular disease, and breast cancer.<sup>15-19</sup> Thus, DHEA may be viewed as a multifunctional steroid hormone. The relevance of these findings in human biology and diseases is perplexing, because humans and nonhuman primates are the only species with the capacity to synthesize and secrete DHEA and DHEAS in quantities surpassing all other known steroids.<sup>20</sup> In light of these considerations, assessments of the potential role of DHEA in human health and disease are of both biologic and clinical importance.

Until recently, limited clinical studies were conducted with mega doses of DHEA, which may induce responses beyond its physiologic action or may, through rapid biotransformation to potent androgens and estrogens, have biologic impact on target tissues, including anabolic effects. In time course studies Mortola and Yen,<sup>21</sup> using a 1,600-mg daily oral dose of DHEA in postmenopausal women (aged 46-61 years) for 4 weeks, demonstrated marked increments of potent androgens and estrogens within 1-2 hours. These increments reached 9-fold for testosterone (T), 20-fold for androstenedione (A) and dihydrotestosterone (DHT), and 2-fold for estrone (E<sub>1</sub>) and estradiol (E<sub>2</sub>) by the 3rd hour after DHEA administration, and levels were sustained during the entire duration of the study. This hyperandrogenic state imposed by a pharmacologic dose of DHEA was associated with a significant decline in sex hormone-binding globulin (SHBG), thyroid-binding globulin, total cholesterol and high density lipoprotein cholesterol, and the appearance of insulin resistance.<sup>21</sup>

#### CELLULAR MECHANISMS OF DHEA DECLINE DURING AGING

Liu *et al.*<sup>22</sup> reported that the progressive decline in DHEA and DHEAS during aging reflected intraadrenal changes in 17 $\alpha$ -hydroxylase enzymatic activities, in that

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a relative deficiency in 17,20 desmolase occurs in aging women, a finding that was recently confirmed in aging men.<sup>23</sup> As 17 $\alpha$ -hydroxylase and 17,20-desmolase are P450 C $\alpha$ 17 enzyme encoded by a single gene,<sup>24</sup> the selective decrease in 17,20-desmolase with unaltered 17 $\alpha$ -hydroxylase activity observed in older individuals suggests a functional shift with aging opposite that seen during adrenarche/puberty when the selective increase in 17,20-desmolase activity leads to a preferential increase in DHEA and DHEAS levels. The mechanism(s) for this switch-on and switch-off of 17,20 desmolase activity during the anabolic state of puberty and the catabolic state of aging remains unclear. The progressive blunting of the ACTH-mediated pulsatile activity of DHEA with advancing age (FIG. 1), without affecting the pulsatile rhythm of cortisol, is highly consistent with a selective intraadrenal biosynthetic defect for DHEA.

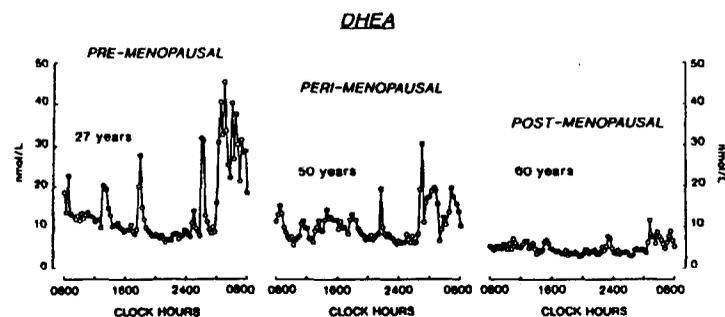


FIGURE 1. Representative 24-hour pulsatile pattern of adrenal DHEA showing a progressive decline with advancing age in pre- and postmenopausal women.

#### REPLACEMENT OF DHEA IN AGING MEN AND WOMEN

We tested the supposition that restoring extracellular levels of DHEA and DHEAS in individuals of advancing age to levels in young adults may have beneficial effects. Because the GH-IGF-I system and immune function decline with aging in parallel to DHEA, we hypothesized that these concomitant changes may be functionally linked. Studies of replacement doses of 50 and 100 mg of DHEA administered orally at bedtime were conducted in men and women aged 40–70 years in double-blind, placebo-controlled cross-over trials of 6- and 12-month durations. In a separate study, we determined the effects of *in vivo* administration of 50 mg DHEA on immune function in aging men.

##### Studies with a 50-Mg Dose of DHEA

##### Oral versus Sublingual Route of Administration

The time course and circulating levels of DHEA and DHEAS after oral versus sublingual routes of administration of 50 mg DHEA in gelatin capsules were

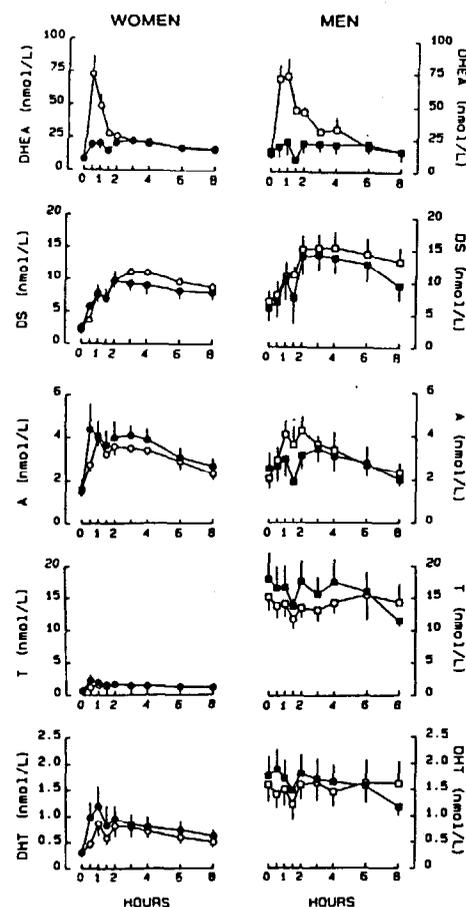


FIGURE 2. Absorption and relative increments of serum levels of DHEA, DHEAS, androstenedione (A), testosterone (T), and dihydrotestosterone (DHT) following oral versus sublingual administration of a 50-mg dose of DHEA in aging men and women (Morales and Yen, unpublished observations).

determined in eight men and eight women. As shown in FIGURE 2, with the exception of serum DHEA levels, the increments over time of circulating DHEAS, A, T, and DHT were rapid and were similar between oral and sublingual routes of administration. DHEA levels, in contrast, showed a rapid (within 30 minutes) and marked elevation lasting for 2 hours after sublingual than after oral administration. Thereafter, all steroid levels, including those of DHEA, were similar with a slight decline towards the end of the experiment at

the 8th hour. Thus, we chose the oral route of administration for subsequent studies because of its ease and reliability of administration in the aging population.

#### Effects of 50-Mg Oral DHEA Replacement (a 6-Month Trial)

A randomized placebo-controlled cross-over trial of nightly oral DHEA administration (50 mg) of 6-months' duration was conducted in 13 men and 17 women 40–70 years of age.<sup>25</sup> During each treatment period, concentrations of androgens, lipids, apolipoproteins, IGF-1, IGF-binding protein-1 (IGFBP-1), and IGFBP-3, insulin sensitivity, percentage of body fat, libido, and sense of well-being were measured. A subgroup of men ( $n = 8$ ) and women ( $n = 5$ ) underwent 24-hour sampling at 20-minute intervals for growth hormone determinations.

DHEA and DHEAS serum levels were restored to those found in young adults<sup>26–28</sup> within 2 weeks of DHEA replacement and were sustained throughout the 3 months of the study. A twofold increase in serum levels of androgens (A, T, and DHT) was observed in women, with only a small rise in A in men. These androgen increments in women remain within the range of young adults. There was no change in circulating levels of SHBG,  $E_1$ , or  $E_2$  in either gender. High density lipoprotein levels declined slightly in women, with no other lipid changes noted for either gender. Insulin sensitivity, determined by euglycemic hyperinsulinemic clamp studies, and percentage of body fat were unaltered. Although mean 24-hour growth hormone and IGFBP-3 levels were unchanged, serum IGF-1 levels increased significantly and IGFBP-1 decreased significantly, resulting in an elevated IGF-1/IGFBP-1 ratio for both genders (FIG. 3), suggesting an increased bioavailability of IGF-1 to target tissues.<sup>29</sup> This was associated with a remarkable increase in perceived physical and psychological well-being for both men (67%) and women (84%) and no change in libido (FIG. 4). These observations and the absence of side effects constitute the first demonstration of novel effects of the replacement dose of DHEA in age-advanced men and women.<sup>25</sup>

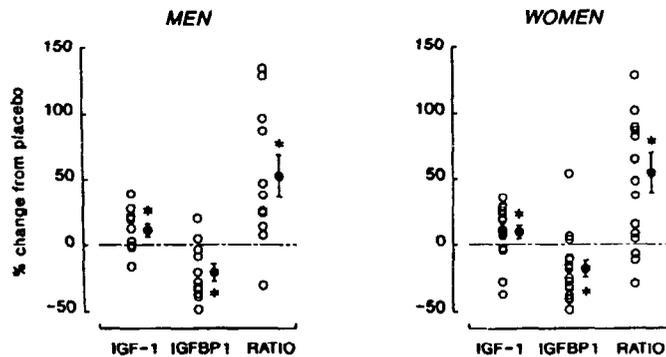


FIGURE 3. Percentage change from placebo of individual values for serum IGF-1, IGFBP-1, and IGF-1/IGFBP-1 ratio after 12 weeks of oral administration of 50 mg DHEA nightly in men and women. The mean  $\pm$  SE of each measurement is also shown. \* $p < 0.05$  compared with placebo (Morales and Yen<sup>21</sup>).

#### One-Year Study of a 100-Mg Dose of DHEA

A randomized double-blind placebo-controlled experiment of 1-year's duration was conducted with a 100-mg oral dose of DHEA or placebo. This study was aimed specifically to assess the effects of doubling the dose and expanding the duration of DHEA administration on *biologic end-points* in aging men ( $n = 8$ ) and women ( $n = 8$ ) 50–65 years of age.

#### Increments of Circulating Steroids

Basal concentrations of all androgenic steroids were either below or near the lower end of the normal range for young adults. Serum DHEAS levels increased several-fold in both men and women at the end of 6 months of DHEA, but not placebo, administration (FIG. 5). These values were near or beyond the upper limit of young adult levels.<sup>26–28</sup> Biotransformation of DHEA in men was limited to a

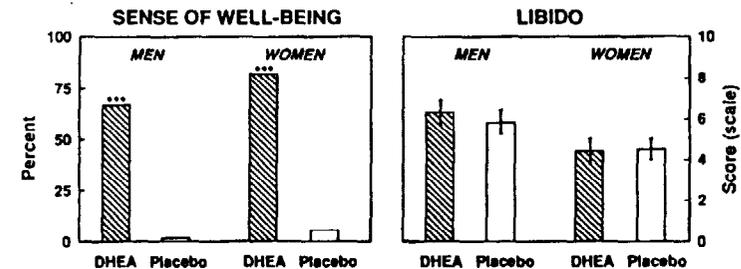


FIGURE 4. Percentage of men and women who self-reported an improved sense of well-being after 12 weeks of oral administration of 50 mg DHEA nightly and after 12 weeks of placebo administration (left panel). \*\*\* $p < 0.005$  compared with placebo values. Scored values of libido on a visual analog scale in men and women after 12 weeks of oral administration of 50 mg DHEA nightly and after 12 weeks of placebo administration are shown. \* $p < 0.01$  compared with opposite gender values.

doubling of A levels. By contrast, there was a three- to fourfold increase in all androgenic steroids (A, T, and DHT) in women, and the levels reached were above the upper limits of normal for adult women. Although SHBG levels were unaltered in men, a 50% decline was seen in women. This gender disparity may be accounted for by the relatively greater increments of androgen levels in women, thereby exerting an inhibitory effect on hepatic production of SHBG. One woman developed facial hair that resolved by the end of the study. Gonadotropin levels in both genders were unaffected by DHEA treatment.

#### Biologic Markers

As seen in the 50-mg dose study, a significant ( $p < 0.05$ ) increase in serum IGF-1 levels occurred in both men and women after 6 months of DHEA treatment at a 100-mg daily dose (FIG. 6). The relative increment in IGF-1 was greater in subjects

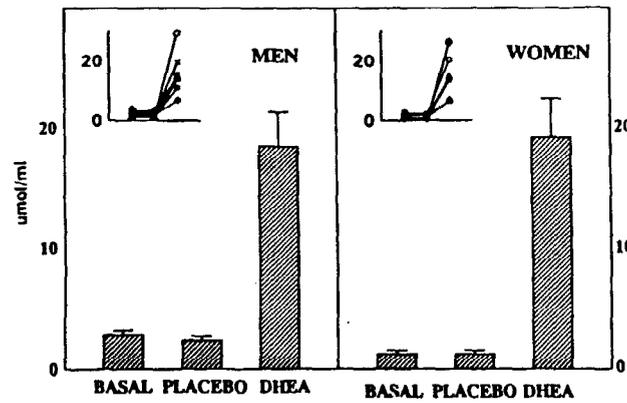


FIGURE 5. Circulating DHEAS levels (mean  $\pm$  SE) at baseline and in response to placebo and DHEA (100 mg/d) administration (6 months each) in aging men ( $n = 8$ ) and women ( $n = 8$ ). (Inset) Changes in individual values.

with low DHEAS levels at baseline. Lean body mass, determined by DEXA, showed an increase in both genders, but significance ( $p < 0.03$ ) was achieved only when both genders were analyzed together (FIG. 7). Knee extension/flexion muscle strength (MedX isometric testing) was increased in men ( $p < 0.01$ ), but not in women, for whom a strong placebo effect was evident (FIG. 8). Lumbar muscle strength was

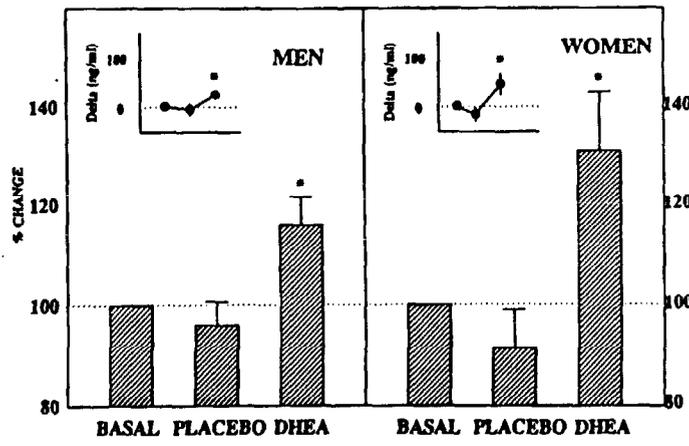


FIGURE 6. Serum IGF-I levels (mean  $\pm$  SE) at baseline (100%) and the percentage change in response to placebo and DHEA (100 mg/d) administration for 6 months in aging men ( $n = 8$ ) and women ( $n = 8$ ). (Inset) Mean  $\pm$  SE increments (delta). \* $p < 0.05$ .

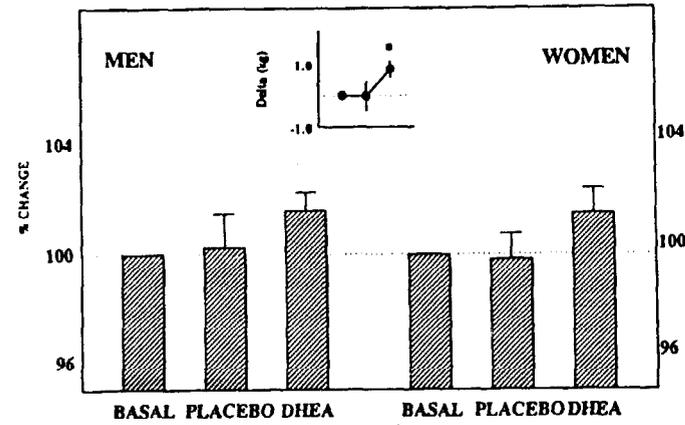


FIGURE 7. Lean body mass (LBM) measured by DEXA at baseline (100%) and the percentage change in response to placebo and DHEA (100 mg/d) administrations in aging men ( $n = 8$ ) and women ( $n = 8$ ). (Inset) Increments (delta). \* $p < 0.05$ .

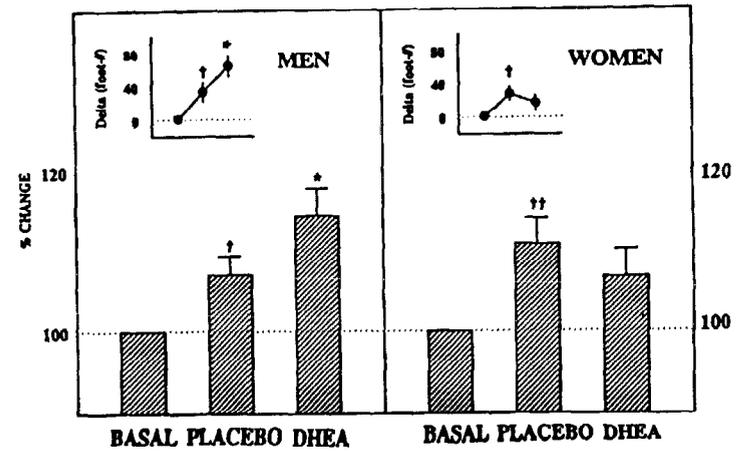


FIGURE 8. Knee extension/flexion muscle strength measured by MedX isometric machine at baseline (100%) and percentage change in response to placebo and DHEA (100 mg/d) in aging men ( $n = 8$ ) and women ( $n = 8$ ) expressed as number of feet. <sup>†</sup> $p < 0.05$  placebo versus baseline; \* $p < 0.05$  DHEA versus placebo. (Inset) Incremental changes (delta).

unaltered. No time course-related changes were noted in muscle strength determinations. Fat body mass (by DEXA) was significantly decreased in men ( $p < 0.05$ ) but not in women (FIG. 9), a finding consistent with that reported by Nestler *et al.*<sup>30</sup> In both genders, no change was noted in lipid profile and apolipoproteins, insulin or glucose levels, nitrogen balance, basal metabolic rate, bone mineral density, or urinary pyridine levels.

In summary, in this extended study (1 year), we have confirmed the ability of DHEA to induce an increase in IGF-1. Furthermore, biologic end-points of increases in lean body mass and muscle strength of the knee were observed. A strong placebo effect was noted in women with regard to muscle strength measurements. A daily dose of 100 mg for 6 months appears to be excessive with respect to the increment of androgens in women and may induce undesirable androgenic effects with time. Thus, a potential gender difference in biotransformation of DHEA and biologic responses requires further study.

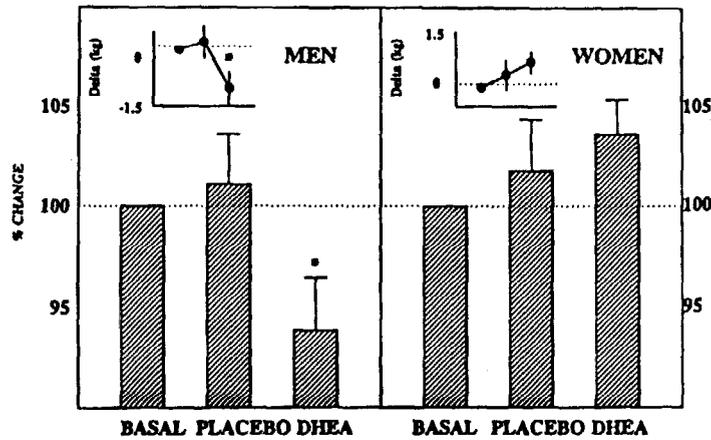


FIGURE 9. Fat body mass (FBM) measured by DEXA at baseline (100%) and the percentage change in response to placebo and DHEA (100 mg/d) for 6 months in aging men ( $n = 8$ ) and aging women ( $n = 8$ ). (Inset) Increments (delta).

**DHEA Administration and Immune Function**

Several lines of evidence derived primarily from animal studies have suggested a role for DHEA in modulating immune function.<sup>8-14</sup> In mice, DHEA administration demonstrated a protective effect against viral induced mortality,<sup>12</sup> and blocked the glucocorticoid-mediated thymocyte destruction *in vivo* and *in vitro*.<sup>13</sup> In a murine model of lupus erythematosus, oral administration of DHEA prevented the formation of antibodies to double-stranded DNA and prolonged survival.<sup>14</sup> *In vitro* studies with both murine<sup>8</sup> and human T cells<sup>9</sup> have shown that DHEA exerts a stimulatory effect on IL-2 secretion, inhibits NK cell differentiation,<sup>31</sup> and prevents the age-related increase in IL-6 production in murine lymphocytes.<sup>10,11</sup> A study of the

therapeutic potential of DHEA (200 mg) in human systemic lupus erythematosus reported an improvement in symptoms, a reduction in corticosteroid requirements,<sup>32</sup> and restoration of impaired IL-2 production by T cells *in vitro*.<sup>33</sup>

To date, the only study examining the *in vivo* effect of a replacement dose of DHEA on human immune function is by Casson *et al.*<sup>34</sup> who reported that in postmenopausal women DHEA treatment with a 50-mg daily oral dose for 3 weeks increased NK cell cytotoxicity and decreased the number of CD4 (T helper) cells, but did not influence *in vitro* IL-6 production. The *in vivo* effects of DHEA treatment on the immune function of elderly men have not been reported.

**DHEA Administration on Immune Function in Men**

A single-blind placebo-controlled trial of 5 months' duration was conducted in nine healthy elderly men who were nonsmokers on no medications, with a mean age of 63.7 years (range 53-69) and mean body mass index of 26.7 kg/m<sup>2</sup> (range 22-30). Subjects took nightly placebo orally for the first 2 weeks followed by oral DHEA (50 mg) for 20 weeks. Fasting blood samples (at 8 AM) were obtained at monthly intervals

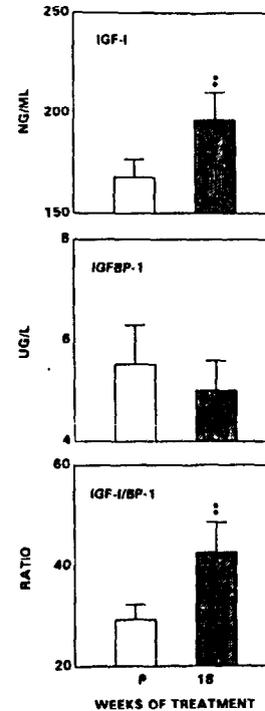


FIGURE 10. Serum levels (mean  $\pm$  SE) of IGF-1, IGFBP-1, and IGF-1/IGFBP-1 ratio in response to placebo and DHEA (50 mg/d) in nine aging men during assessments of immune function. \*\* $p < 0.01$ .

TABLE 1. Effect of DHEA on Lymphocyte Subsets as Determined by Flow Cytometry\*

Cell Marker	Weeks of Treatment						
	P	2	6	10	14	18	20
CD20 (B cells)	9.5 ± 2.1	14.5 ± 2.7*	9.5 ± 1.9	14.1 ± 3*	11.4 ± 2.8	11.4 ± 2.7	13.9 ± 4.0
CD14 (monocytes)	11.6 ± 1.9	21.2 ± 3.5**	14.0 ± 2.5	14.2 ± 2.1	14.7 ± 2.6	16.2 ± 2.9	17.9 ± 2.9**
CD3 (T cells)	68.4 ± 2.8	66.7 ± 4.3	61.7 ± 3.8	65.4 ± 3.1	69.8 ± 2.7	68.7 ± 3.7	69.1 ± 4.0
CD4 (T helper)	44.2 ± 3.4	39.4 ± 4.7	39.8 ± 2.9	40.5 ± 3.1	44.6 ± 2.4	43.9 ± 3.6	41.3 ± 4.1
CD8 (T suppressor)	32.7 ± 3.7	33.0 ± 3.0	31.2 ± 3.1	35.4 ± 3.1	33.6 ± 2.9	36.2 ± 1.9	38.3 ± 3.8
TCR α/β	59.2 ± 2.5	55.9 ± 2.9	42.6 ± 5.8	57.0 ± 3.2	51.9 ± 3.1	61.7 ± 3.9	62.0 ± 5.8
TCR γ/δ	5.7 ± 1.1	6.2 ± 0.97	6.2 ± 1.2	7.4 ± 1.1	7.7 ± 1.2	10.9 ± 1.2	10.5 ± 1.1**
CD25 (IL-2 receptor)	9.4 ± 1.2	6.7 ± 1.7	4.8 ± 0.83*	10.6 ± 2.3	11.7 ± 2.7	15.1 ± 1.5*	12.4 ± 2.4*
CD57 (NK)	24.1 ± 3.5	23.3 ± 2.9	24.0 ± 3.3	26.0 ± 2.4	22.8 ± 3.6	30.9 ± 2.9**	31.5 ± 3.2**
CD16 (NK)	15.4 ± 1.9	13.8 ± 1.4	18.1 ± 1.9	18.2 ± 2.5	15.3 ± 1.7	19.9 ± 2.2	21.0 ± 2.7*

\*Values are expressed as % lymphocytes ± SEM.

\**p* < 0.05; \*\**p* < 0.01 versus placebo (P).

for assessment of immune function and determination of serum levels of IGF-I and IGFBP-1.

Our data show that DHEA treatment significantly (*p* < 0.01) elevated serum IGF-1 levels with a decreasing trend for IGFBP-1 levels resulting in a significant (*p* < 0.01) elevation in the IGF-1/IGFBP-1 ratio (FIG. 10), a finding confirming our previous studies. The effect of *in vivo* DHEA treatment on lymphocyte subsets as determined by flow cytometry is shown in TABLE 1. In response to DHEA treatment a biphasic increase (*p* < 0.01) in monocytes (CD14) at 2 and 20 weeks was found. B cells (CD20) showed a fluctuating pattern with transient increases at 2 and 10 weeks (*p* < 0.05) followed by a rise (nonsignificant) at 20 weeks. Functional activation of B cells occurred as evidenced by a dose-related increase in proliferative response to the B-cell-specific mitogen pokeweed at 12 and 20 weeks (TABLE 2A and B), a response pattern parallel that seen in B-cell number. Serum IgG, IgM, and IgA were not affected (TABLE 2B). DHEA treatment did not affect basal levels of IL-6 production, but it enhanced phytohemagglutinin stimulated IL-6 production at 20 weeks (FIG. 11).

The number of total T lymphocytes (CD3) and T-cell subsets (CD4, CD8) was unaffected by DHEA treatment. However, there was a doubling in the number of T cells expressing the T-cell receptor γ/δ (TCR γ/δ) (*p* < 0.01) by 20 weeks of treatment, but not the α/β receptor (TCR α/β) (TABLE 1). T-cell function was activated as evidenced by an increased proliferative response to the T-cell-specific mitogen phytohemagglutinin (0.1 μg/ml) by 12 weeks (TABLE 2). This was accompanied by a significant (*p* < 0.01) rise in serum sIL-2R (measured by ELISA, Genzyme, Boston, Massachusetts) by 12 weeks, T cells expressing the IL-2 receptor (CD25), and the enhanced phytohemagglutinin-induced secretion of IL-2 (measured by ELISA, Bisource, Camarillo, California) by 20 weeks (FIG. 12). However, a transient decrease (*p* < 0.05) in IL-2R occurred at 6 weeks. The significance of this finding is unclear. In addition, DHEA treatment significantly (*p* < 0.01) increased NK cell number (CD16, CD57) by 18–20 weeks with a parallel rise in cytotoxicity (*p* < 0.01) as determined by a Cr-51 release assay using the K-562 cell line as the target<sup>35</sup> (FIG. 13).

TABLE 2A. Effect of DHEA Treatment on Lymphocytes Response to Mitogens\*

	Weeks of Treatment				
	Baseline	Placebo	4	12	20
Pokeweed mitogen					
0.5 μg/ml	0.84 ± 0.17	1.0 ± 0.21	1.3 ± 0.18	2.1 ± 0.41*	2.1 ± 0.45*
5 μg/ml	2.2 ± 0.43	2.3 ± 0.50	2.5 ± 0.42	3.8 ± 0.64	5.3 ± 1.4**
Phytohemagglutinin					
0.1 μg/ml	1.3 ± 0.14	1.2 ± 0.13	1.7 ± 0.25	2.2 ± 0.50*	1.2 ± 0.12
2 μg/ml	6.2 ± 2.3	5.9 ± 2.3	5.9 ± 1.1	8.5 ± 2.3	5.3 ± 1.2

\*Values are expressed as stimulation index (cpm in treatment wells/cpm in test wells) ± SEM.

\**p* < 0.05 versus placebo.

TABLE 2B. Effect of DHEA Treatment on Circulating Immunoglobulins

Immunoglobulins	Weeks of Treatment			
	Placebo	2	10	20
IgG (mg/l)	11,152 ± 757	11,015 ± 515	10,933 ± 675	11,245 ± 860
IgA (mg/l)	1,869 ± 168	1,934 ± 191	1,860 ± 192	1,914 ± 174
IgM (mg/l)	1,216 ± 208	1,187 ± 187	1,223 ± 202	1,259 ± 209

Our study demonstrates a time-related stimulatory effect of DHEA on the immune function of aging men. Peripheral lymphocytes appear to be targets of DHEA, with most effects occurring with a latent phase of 10–12 weeks. These results are in accord with the *in vitro* animal data showing a stimulatory effect of DHEA on IL-2 production,<sup>11,12</sup> but in addition we have demonstrated an increase in

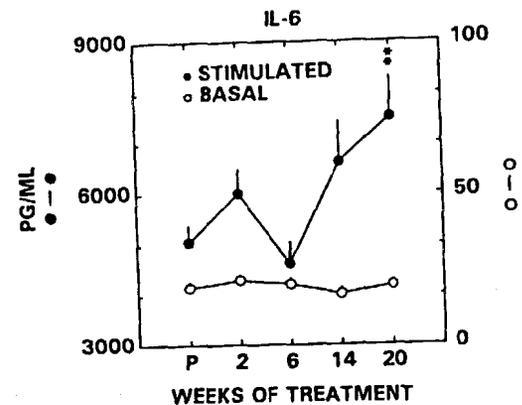


FIGURE 11. Concentrations of IL-6 (mean ± SE) in cultured lymphocytes under unstimulated (○—○) and phytohemagglutinin-stimulated (20 μg) conditions determined during placebo and during DHEA (50 mg/d) treatment. \*\**p* < 0.01.

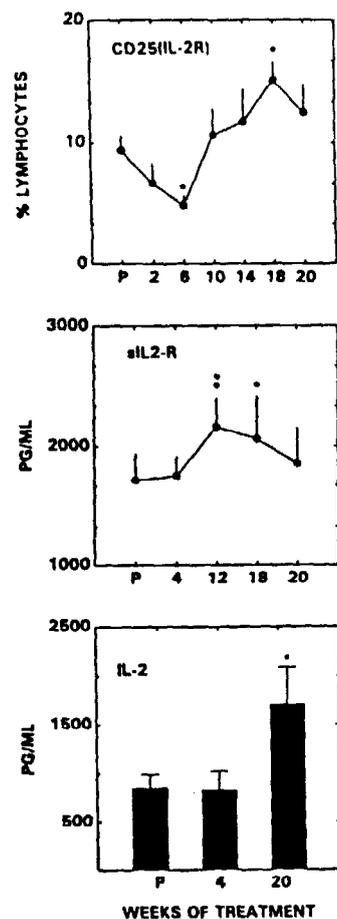


FIGURE 12. Percentage of lymphocytes expressing IL-2 receptors (*top*), concentrations of sIL-2 receptors in serum (*middle*), and IL-2 in culture (*bottom*) during placebo (P) and during DHEA (50 mg/d) treatment. \* $p < 0.05$ ; \*\* $p < 0.01$ .

cells expressing IL-2 receptor and sIL-2 receptor in serum. In contrast with murine data showing inhibition of an age-related increase in IL-6 by DHEA,<sup>13</sup> we found an unaltered low level of basal secretion of IL-6, but augmented phytohemagglutinin-stimulated IL-6 production in response to DHEA treatment in men. In contrast with data obtained in postmenopausal women,<sup>28</sup> we did not observe a decrease in CD4<sup>+</sup> T cells. However, a similar increase in NK cells was found with a difference in time course of activation (3 vs 18 weeks, respectively) in postmenopausal women and in our current study in men. The mechanism(s) by which DHEA exerts its lymphocyto-

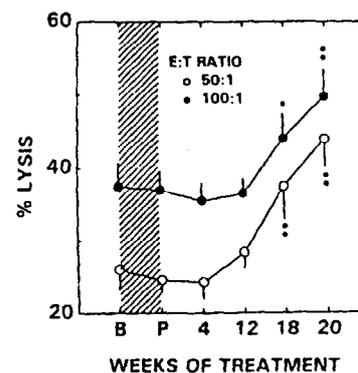


FIGURE 13. Percentage of lysis of the target cells at two effector/target (E:T) ratios at baseline (B) following placebo (P) and DHEA treatments. \* $p < 0.05$ ; \*\* $p < 0.01$ .

tropic effects is unknown. The temporal synchrony of the increase in circulating IGF-1 and immune activation by DHEA suggests that the immunoenhancing effects of DHEA may be mediated by IGF-1 by virtue of its immune regulating properties, which have been demonstrated both *in vivo* and *in vitro*.<sup>36,37</sup> The question as to just how these findings translate to immunity against foreign antigens is being addressed in ongoing studies.

#### SUMMARY

DHEA in appropriate replacement doses appears to have remedial effects with respect to its ability to induce an anabolic growth factor, increase muscle strength and lean body mass, activate immune function, and enhance quality of life in aging men and women, with no significant adverse effects. Further studies are needed to confirm and extend our current results, particularly the gender differences.

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# Activation of Immune Function by Dehydroepiandrosterone (DHEA) in Age-Advanced Men

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**Background.** Substantial data from animal studies have demonstrated a stimulatory effect of dehydroepiandrosterone (DHEA) on immune function. However, little is known about the effects of DHEA on the human immune system. Since aging is associated with a decline in immune function and in DHEA production, we proposed that oral administration of DHEA to elderly men would result in activation of their immune system.

**Methods.** Nine healthy age-advanced men (mean age of 63 years) with low DHEA-sulfate levels participated in this study. They were treated nightly with an oral placebo for 2 weeks followed by DHEA (50 mg) for 20 weeks. Fasting (0800h-0900h) blood samples were obtained at 4- to 8-week intervals for immune function studies and hormone determinations. Freshly isolated peripheral lymphocytes were used for flow cytometric identification of lymphocyte subsets, cells expressing the IL-2 receptor (IL-2R), mitogen stimulation studies, and for determining natural killer (NK) cell number and cytotoxicity. Levels of interleukin-2 (IL-2) and IL-6 secreted from cultured lymphocytes were determined under basal and mitogen stimulated conditions. Sera were analyzed for soluble IL-2 Receptor (sIL-2R) levels, insulin-like growth factor-1 (IGF-I) and IGF binding protein-1 (IGFBP-1) concentrations.

**Results.** Baseline levels of serum DHEA sulfate (DHEAS), a stable marker of circulating DHEA levels, were 2 standard deviations below young adult values and increased 3-4 fold within 2 weeks. These levels were sustained throughout the duration of DHEA administration. When compared with placebo, DHEA administration resulted in a 20% increase ( $p < .01$ ) in serum IGF-I, a decreasing trend in IGFBP-1, and a 32% increase in the ratio of IGF-I/IGFBP-1 ( $p < .01$ ). Activation of immune function occurred within 2-20 weeks of DHEA treatment. The number of monocytes increased significantly ( $p < .01$ ) after 2 (45%) and 20 (35%) weeks of treatment. The population of B cells fluctuated with increases ( $p < .05$ ) at 2 (35%) and 10 (29%) weeks of treatment. B cell mitogenic response increased 62% ( $p < .05$ ) by 12 weeks unaccompanied by changes in serum IgG, IgA, and IgM levels. Total T cells and T cell subsets were unaltered. However, a 40% increase ( $p < .05$ ) in T cell mitogenic response, 39% increase in cells expressing the IL-2R (CD25<sup>+</sup>) ( $p < .05$ ), and 20% increase in serum sIL-2R levels ( $p < .01$ ) were found at 12-20 weeks of DHEA treatment, suggesting a functional activation of T lymphocytes occurred. In vitro mitogen stimulated release of IL-2 and IL-6 was enhanced 50% ( $p < .05$ ) and 30% ( $p < .01$ ) respectively by 20 weeks of treatment without basal secretion being affected. NK cell number showed a 22-37% increase ( $p < .01$ ) by 18-20 weeks of treatment with a concomitant 45% increase ( $p < .01$ ) in cytotoxicity. There were no adverse effects noted with DHEA administration.

**Conclusion.** Administration of oral DHEA at a daily dose of 50 mg to age-advanced men with low serum DHEAS levels significantly activated immune function. The mechanism(s) to account for the immunoenhancing properties of DHEA are unclear. Consideration is given to the potential role of an increase in bioavailable IGF-I, which by virtue of its mitogenic effects on immune cell function, may mediate the DHEA effects. While extended studies are required, our findings suggest potential therapeutic benefits of DHEA in immunodeficient states.

IT is well recognized that the immune system performs biologic functions that are indispensable to survival in mammalian species. Immunologic competence is largely dependent on both the ability and rapidity of response to antigens by specific cellular elements (1). Aging is associated with a decline in immune function that leads to an increased incidence of infection, cancer, and autoimmune disease (2). Age-related changes in immunity primarily involve alterations in T cell function, including a decreased proliferative response of T cells to mitogens (3,4), diminished IL-2 production and expression of interleukin-2 receptor (IL-2R) (5). Altered B cell function also occurs with aging, as evidenced by a decreased ability to generate antibodies to antigens and decreased effectiveness of vaccines to confer immunity. In contrast to T and B cells, the number and activity of natural killer (NK) cells have been

reported to either increase (6,7) or remain the same (8) in the aging population.

Major research efforts have focused on finding means to enhance immune function in immunodeficiency states. Based primarily on evidence derived from studies of the murine model, dehydroepiandrosterone (DHEA) and its sulfated ester (DHEAS) have been shown to have immunoenhancing properties. DHEA reverses the corticosteroid and stress-induced inhibition of immune function (9). The effects of DHEA on immune function and its antagonism of corticosteroid effects have been recently reviewed (10-12). In mice, DHEA administration by various routes was shown to have a protective effect against systemic Coxsackie virus B4 and herpes simplex type 2 encephalitis (13) and to block the glucocorticoid mediated thymocyte involution in vivo and in vitro (14). In a murine model for lupus erythemato-

sus. pharmacologic doses of oral DHEA prevented the formation of circulating auto-antibodies to double-stranded DNA in plasma, prolonged survival (15), and reversed an IL-2 deficient state (16). In vitro studies have shown that DHEA exerts a stimulatory effect on IL-2 secretion, inhibits NK cell differentiation (17), and prevents the age-related increase in IL-6 production (18). In an elegant series of in vivo experiments, Daynes and associates have demonstrated the ability of DHEA, but not DHEAS, to enhance IL-2 production by activated murine T cells (19,20). Araneo et al. found that treatment of burned mice with a single injection of 100 µg of DHEA sc restored their capacity to produce T cell derived lymphokines and to generate a cellular immune response (21). The same group also demonstrated that topical application of DHEA (10 µg) or sc injection (100 µg) of DHEAS in aging mice corrected the age-associated dysregulated production of T cell lymphokines from various lymphoid organs (22). When DHEA was incorporated into the hepatitis vaccine, the combination produced an enhanced antibody response against hepatitis B surface antigen (22). DHEA has also been shown to increase IL-2 production and cytotoxic effector function in human T cells (23) and inhibit Epstein-Barr virus induced morphologic transformation and stimulation of DNA synthesis in human lymphocytes in vitro (24).

Human studies examining DHEA effects on immune function are limited. In a prospective randomized double-blind study, Casson et al. reported that daily treatment of postmenopausal women with oral DHEA (50 mg) for 3 weeks increased NK cell cytotoxicity, decreased the number of CD4 (T helper) cells, and inhibited T cell mitogenic response without affecting IL-6 or IL-2 secretion (25). In a Phase I open label study of patients with symptomatic HIV infection, mega oral doses of DHEA (750–2250 mg/day) for 16 weeks failed to induce improvement in immune parameters (26). The therapeutic potential of DHEA in systemic lupus erythematosus (SLE) was tested in an open label, non-controlled trial involving 10 women with mild to moderate disease; oral DHEA (200 mg daily dose) produced a significant symptomatic improvement and decreased their corticosteroid requirements (27). In another study, patients with SLE were found to have very low serum levels of DHEA and administration of DHEA restored the impaired IL-2 production of their T cells in vitro (28). In light of these findings and the lack of information on the effects of DHEA on immune function in age-advanced men we have conducted the following studies.

## METHODS

### Subjects

Nine healthy age-advanced men, nonsmokers, on no medications, with a mean age of 63.7 years (range 53–69) and body mass index of 26.7 kg/m<sup>2</sup> (range 22–30) were recruited for this study. Psychiatric and concurrent medical illness and major depression were excluded by history, physical examination, blood chemistry, complete blood count, and Beck depression tests. The protocol was approved by Human Subjects Committee of the University of California, San Diego.

### Study Design and Protocol

The study design was a single-blind placebo-controlled trial of 22 weeks duration. Subjects took an oral placebo (50 mg) nightly for the first 2 weeks followed by oral DHEA (50 mg) for 20 weeks. Subjects were instructed not to change their dietary habits or exercise regimen.

Subjects were seen as outpatients at 4- and 8-week intervals. During each visit they filled out an open-ended questionnaire regarding self-reported changes and side effects. Fasting blood samples were drawn between 0800h–0900h for immune studies, hormone measurements, and standard complete blood and chemistry panels. Blood pressure and body weight were monitored at each visit.

### Hormone Measurements

All hormone measurements for each individual subject were performed in duplicate in the same assay. DHEAS and cortisol were measured by a specific RIA previously described (29). IGF-I was measured by acid-ethanol extraction by a RIA kit (Nichols Institute, San Juan Capistrano, CA). IGFBP-1 was measured by a time-resolved immunofluorometric assay (30). Assay sensitivities were 0.6 µmol/L, 13.5 ng/ml, 0.1 µg/L and 20 nmol/L for DHEAS, IGF-I, IGFBP-1, and cortisol respectively. Inter- and intraassay coefficients of variation for DHEAS were 5% and 7%. IGF-I were 8% and 3%, IGFBP-1 were 8% and 6%, and cortisol were 6.4 and 5.4 respectively.

### Immune Cell Studies

Lymphocytes were isolated from freshly drawn blood by Ficoll-Hypaque centrifugation (Sigma, St. Louis, MO) (31). Cells were then washed three times with PBS buffer, counted in a hemocytometer, and suspended in the appropriate concentration in RPMI 1640 containing 1% glutamine, 1% penicillin-streptomycin and 10% fetal calf serum.

**Mitogen assays.** — Lymphocytes were incubated at a concentration of  $1 \times 10^6$  cell/ml in the presence of PHA (Murex Diagnostics, Norcross, GA) at 0.1 and 2 µg/ml, and PWM (Gibco, Gaithersburg, MD) at 0.5 and 5 µg/ml, in 5% CO<sub>2</sub> at 37 °C for 6 days at which time 0.2 µCi of <sup>3</sup>H-thymidine was added. The cells were harvested 18 hours later onto filters and counted in a liquid scintillation counter. Control wells contained only buffer. Results were expressed in terms of stimulation index determined as follows: *cpm in mitogen containing wells/cpm in control wells*.

**Flow cytometry.** —  $2 \times 10^6$  cells were incubated with monoclonal antibodies (Beckton-Dickinson, San Jose, CA) against various cell surface antigens listed in Table 1. After 20 minutes of incubation at 4 °C the cells were washed two times with PBS buffer and fixed with 2% paraformaldehyde. Cells were then analyzed in a FACS scan flow cytometer (Beckton-Dickinson Immunocytometry System, San Jose, CA), and data were analyzed by Lysys II software.

**NK cell activity.** — Target K-562 cells were incubated with 200 µCi of chromium-51 for 2 hours in 5% CO<sub>2</sub> at 37 °C, after which cells were washed and adjusted to 10<sup>6</sup> cells per ml. Target cells were then incubated with lymphocytes

isolated from the DHEA treated subjects at effector to target (E:T) ratios of 50:1 and 100:1 in 5% CO<sub>2</sub> at 37 °C for 4 hours. One hundred µl of the well contents was counted in a gamma counter. Target cells were incubated in culture medium with and without 3% SDS for measurement of spontaneous and maximum release. The specific lysis was determined as follows: (experimental release)-(spontaneous release)/(maximum release)-(spontaneous release).

**Cytokine secretion and immunoglobulins.** —  $1 \times 10^6$  cells/ml were incubated with and without PHA (20 µg/ml) for 48 hours in 5% CO<sub>2</sub> at 37 °C. After 48 hours the well contents were centrifuged at 600 g for 5 minutes and the supernatant stored at -70 °C for subsequent measurements of IL-2 and IL-6 by ELISA kits (Biosource, Camarillo, CA). The sensitivity of these assays was 8.7 pg/ml and 2 pg/ml, respectively. sIL-2R was measured by ELISA (Genzyme, Boston, MA) with a sensitivity of 100 pg/ml. Immunoglobulins were measured by radial immunodiffusion (The Binding Site, La Jolla, CA).

#### Statistics

The endocrine data were analyzed by paired Student's *t*-test. All other data were analyzed by ANOVA with repeated measure. Post hoc testing was performed by the method of least square deviation. All time points following the 20 weeks of DHEA treatment were compared with baseline and placebo treatment values. Significance was established at  $p < .05$ .

#### RESULTS

Baseline DHEAS levels including those from a 53-year-old subject were two standard deviations below the young adult range. DHEA administration restored DHEAS levels to young adult levels (32) within 2 weeks and sustained through the duration of the study (Table 2). Serum cortisol levels were unaltered by DHEA administration and thus the DHEAS/cortisol ratio increased 4-fold (Table 2). When compared to baseline or placebo, DHEA treatment was associated with a significant ( $p < .01$ ) elevation in serum IGF-I levels and a decreasing trend in IGFBP-1 levels, resulting in a significant ( $p < .01$ ) increase of the IGF-I/IGFBP-1 ratio (Figure 1). This finding confirmed our earlier studies and suggested an increase in bioavailability of IGF-I to target cells (33). The effect of ex vivo DHEA treatment on lymphocyte subsets is shown in Table 1. In response to DHEA treatment, the number of monocytes (CD14) increased at 2 and 20 weeks ( $p < .01$ ). B cell numbers (CD20) showed a fluctuating pattern with a transient rise at 2 weeks ( $p < .01$ ) and an increase by 20 weeks ( $p < .01$ ). This increase was accompanied by functional activation, as evidenced by a dose-related increase in proliferative response to PWM at 12 weeks with further increases by 20 weeks (Figure 2). Serum IgG, IgM, and IgA levels were not affected over the course of study (Table 3). Although DHEA treatment did not affect the low levels of basal IL-6 secretion, PHA-induced secretion of IL-6 increased significantly ( $p < .01$ ) by 20 weeks (Figure 3).

The number of total T lymphocytes (CD3) and T cell subsets (CD4, CD8) were unaffected by DHEA treatment.

Table 1. Effect of DHEA on Lymphocyte Subsets and Monocytes as Determined by Flow Cytometry: Values Are Expressed as % Lymphocytes  $\pm$  SEM

Cell Marker	Placebo	Weeks of Treatment					
		2	6	10	14	18	20
CD20 (B cells)	17.9 $\pm$ 2.2	27.7 $\pm$ 3.6**	18.0 $\pm$ 2.3	18.6 $\pm$ 2.4	19.8 $\pm$ 2.5	20.5 $\pm$ 2.7	25.2 $\pm$ 2.7**
CD14 (monocytes)	11.6 $\pm$ 1.9	21.2 $\pm$ 3.5**	14.0 $\pm$ 2.5	14.2 $\pm$ 2.1	14.7 $\pm$ 2.6	16.2 $\pm$ 2.9	17.9 $\pm$ 2.9**
CD3 (T cells)	68.4 $\pm$ 2.8	66.7 $\pm$ 4.3	61.7 $\pm$ 3.8	65.4 $\pm$ 3.1	69.8 $\pm$ 2.7	68.7 $\pm$ 3.7	69.1 $\pm$ 4.0
CD4 (T helper)	44.2 $\pm$ 3.4	39.4 $\pm$ 4.7	39.8 $\pm$ 2.9	40.5 $\pm$ 3.1	44.6 $\pm$ 2.4	43.9 $\pm$ 3.6	41.3 $\pm$ 4.1
CD8 (T suppressor)	32.7 $\pm$ 3.7	33.0 $\pm$ 3.0	31.2 $\pm$ 3.1	35.4 $\pm$ 3.1	33.6 $\pm$ 2.9	36.2 $\pm$ 1.9	38.3 $\pm$ 3.8
TCR $\alpha/\beta$	59.2 $\pm$ 2.5	55.9 $\pm$ 2.9	42.6 $\pm$ 5.8	57.0 $\pm$ 3.2	51.9 $\pm$ 3.1	61.7 $\pm$ 3.9	62.0 $\pm$ 5.8
TCR $\gamma/\delta$	5.7 $\pm$ 1.1	6.2 $\pm$ 0.97	6.2 $\pm$ 1.2	7.4 $\pm$ 1.1	7.7 $\pm$ 1.2	10.9 $\pm$ 1.2	10.5 $\pm$ 1.1**
CD25 (IL-2 receptor)	9.4 $\pm$ 1.2	6.7 $\pm$ 1.7	4.8 $\pm$ 0.83*	10.6 $\pm$ 2.3	11.7 $\pm$ 2.7	15.1 $\pm$ 1.5*	12.4 $\pm$ 2.4*
CD57 (NK)	24.1 $\pm$ 3.5	23.3 $\pm$ 2.9	24.0 $\pm$ 3.3	26.0 $\pm$ 2.4	22.8 $\pm$ 3.6	30.9 $\pm$ 2.9**	31.5 $\pm$ 3.2**
CD16 (NK)	19.5 $\pm$ 1.9	17.9 $\pm$ 2.4	19.6 $\pm$ 1.9	24.0 $\pm$ 2.0	21.0 $\pm$ 2.7	31.0 $\pm$ 2.8**	28.6 $\pm$ 3.1**

\* $p < .05$ ; \*\* $p < .01$  vs placebo.

Table 2. Serum Concentrations (mean  $\pm$  SE) of DHEAS ( $\mu$ mol/L), Cortisol (nmol/L) and the Ratio of DHEAS/Cortisol at Baseline and After Treatment With Placebo and DHEA (50 mg/day)

	Baseline	Placebo	Weeks of Treatment				
			2	10	14	18	20
DHEAS ( $\mu$ mol/L)	3.15 $\pm$ 0.39	3.22 $\pm$ 0.39	11.8 $\pm$ 1.07	10.9 $\pm$ 1.12	10.7 $\pm$ 1.44	12.1 $\pm$ 0.81	12.9 $\pm$ 1.13
Cortisol (nmol/L)	273 $\pm$ 28.8	283 $\pm$ 25.1	282 $\pm$ 37.3	238 $\pm$ 26.3	246 $\pm$ 25.1	231 $\pm$ 31.3	245.0 $\pm$ 36.3
DHEAS/cortisol	13.0 $\pm$ 2.2	11.9 $\pm$ 1.7	48.2 $\pm$ 6.4	49.2 $\pm$ 7.6	44.4 $\pm$ 6.9	56.3 $\pm$ 6.7	53.0 $\pm$ 6.7

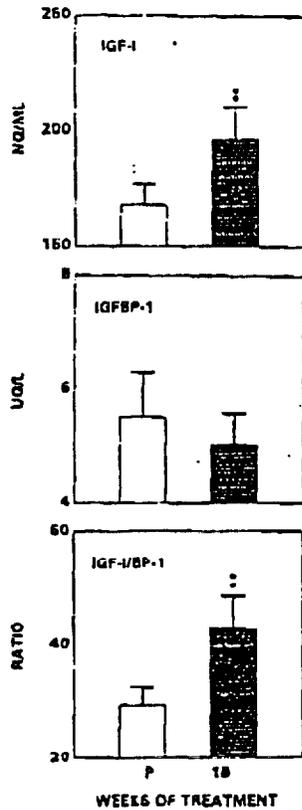


Figure 1. Serum levels (mean  $\pm$  SE) of IGF-I, IGFBP-1 and IGF-I/IGFBP-1 ratio in response to placebo (P) and DHEA treatment in 9 aging men. \*\* $p < .01$ .

However, there was a doubling in the number of T cells expressing the T cell receptor  $\gamma/\delta$  (TCR  $\gamma/\delta$ ) ( $p < .01$ ) but not the TCR  $\alpha/\beta$  by 20 weeks of treatment (see Table 1). T cell function was activated as evidenced by an increased proliferative response to PHA (0.1  $\mu\text{g/ml}$ ) within 12 weeks (Figure 2). This was accompanied by an increase in T cells expressing the IL-2R (CD25) by 18 weeks ( $p < .05$ ) (Table 1 and Figure 4), and enhanced PHA-induced IL-2 secretion by 20 weeks (Figure 4). Serum sIL-2R levels also increased significantly ( $p < .01$ ) by 12 weeks (Figure 4). Moreover, DHEA treatment led to a significant ( $p < .01$ ) increase in NK cell number (CD16, CD57) by 18–20 weeks (Table 1). This was accompanied by a parallel increase in their cytotoxicity at both high and low E:T ratios ( $p < .01$ ) (Figure 5).

DHEA was well tolerated without significant effects on blood pressure, body weight, blood count, or chemistry profile.

#### DISCUSSION

Our study demonstrates the stimulatory effects of DHEA on the immune function of age-advanced men. These effects of DHEA appear to require a latent phase of several weeks. This delay in the immune response to DHEA is to be expected as changes in immune function occur at a slower pace in aging populations (34). The *ex vivo* evidence pre-

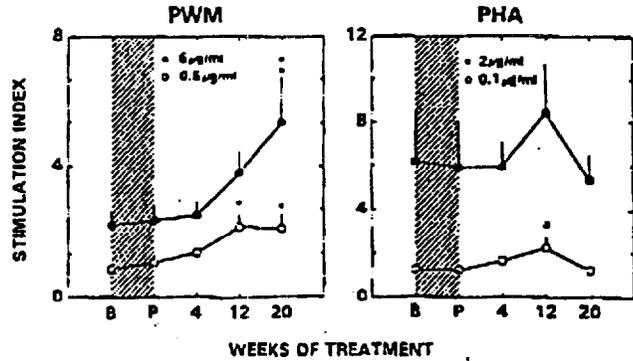


Figure 2. Response of lymphocytes from subjects at baseline (B), following placebo (P) and DHEA treatment to the B cell mitogen PWM, and the T cell mitogen PHA. \* $p < .05$ , \*\* $p < .01$ .

Table 3. Effect of DHEA Treatment on Circulating Immunoglobulins; Values are mean  $\pm$  SEM

	Placebo	Weeks of Treatment		
		2	10	20
IgG (mg/L)	11152 $\pm$ 757	11015 $\pm$ 515	10933 $\pm$ 675	11245 $\pm$ 860
IgA (mg/L)	1869 $\pm$ 168	1934 $\pm$ 191	1860 $\pm$ 192	1914 $\pm$ 174
IgM (mg/L)	1216 $\pm$ 208	1187 $\pm$ 187	1223 $\pm$ 202	1259 $\pm$ 209

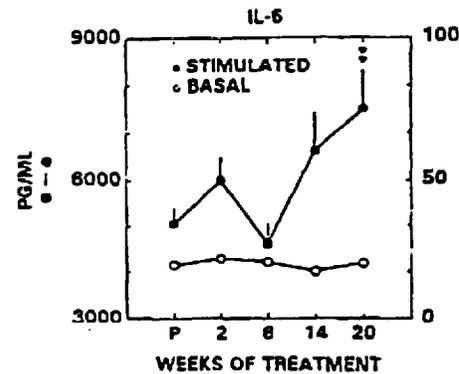


Figure 3. Concentration of IL-6 (mean  $\pm$  SE) in media from cultured lymphocytes under unstimulated and PHA-stimulated (20  $\mu\text{g/ml}$ ) conditions determined following placebo (P) and DHEA treatment. \*\* $p < .01$ .

sented here confirms and extends animal-derived data concerning the effects of DHEA on T cell function, and provides novel B cell findings. DHEA treatment rejuvenated the immune system by increasing the secretion of IL-2, a potent T cell growth factor, increasing the number of cells expressing the IL-2R (CD25), inducing a rise in serum sIL-2R, and enhancing T cell responsiveness to mitogen stimulation, all of which decline during physiologic aging (34). These findings suggest that activation of T cells expressing IL-2R may permit the growth-promoting effects of IL-2 on T cells via an autocrine mode of action. In addition to serving as a growth factor to T lymphocytes, IL-2 may also stimulate the prolif-

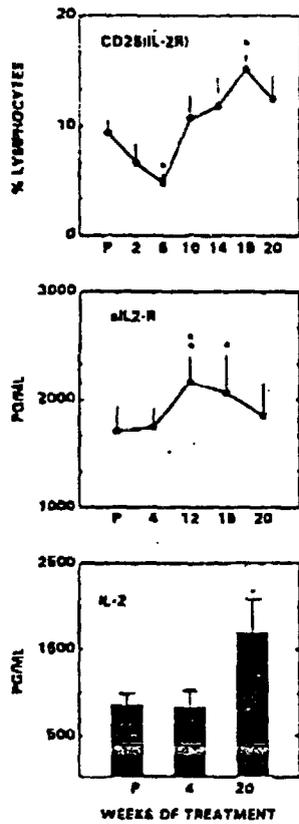


Figure 4. Top panel depicts the % lymphocytes expressing the IL-2R (CD25) as determined by flow cytometry. Middle panel shows circulating sIL-2R as determined by ELISA. Lower panel shows the concentration of IL-2 as determined by ELISA in media from cultured PHA stimulated (20  $\mu$ g/ml) lymphocytes following placebo and DHEA treatment. \*\* $p < .01$ .

eration of NK cells and enhance their cytolytic function via a paracrine mode of action (35).

Moreover, IL-2 may also act on human B cells both as a growth factor and as a stimulus for antibody synthesis (36-37). The abundance of IL-2 synthesized by activated CD4<sup>+</sup> T cells is viewed as an important determinant of the magnitude of immune response. Thus, the observed increases in IL-2 and IL-2R constitute a major attribute of DHEA treatment in enhancing the immune function of age-advanced men. The significance of an increase in cells expressing the T cell receptor  $\gamma\delta$  is unknown. Since TCR  $\gamma\delta^+$  cells reside predominantly in epithelial surfaces (38), DHEA may play a role in mucosal immunity. DHEA administration also activated B cell function as evidenced by the increased number and responsiveness of these cells to mitogen stimulation, an event which may be related to the trophic effect of IL-2. These findings suggest that DHEA treatment of age-advanced men, in an appropriate dose (e.g., 50 mg), may lead to an enhanced antibody response.

Normal aging in human beings is associated with increased basal IL-6 production by lymphocytes (18,39), whereas stimulated IL-6 secretion is either not affected (18) or reduced (40). Dysregulation of this cytokine with increased basal secretion has been proposed to contribute to

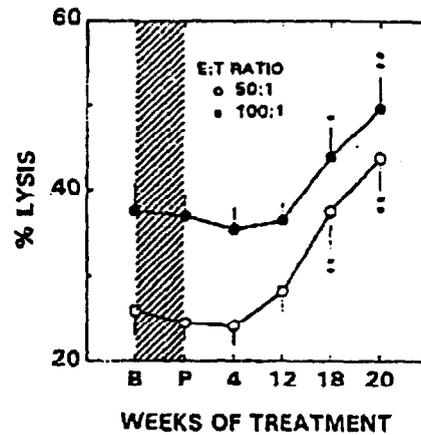


Figure 5. NK cell cytotoxicity determined at two effector/target (E:T) ratios following placebo (P) and DHEA treatment. \* $p < .05$ ; \*\* $p < .01$ .

the pathogenesis of age-associated diseases such as B cell lymphoma, osteoporosis, and Alzheimer's disease (41). In our study, basal secretion of IL-6 was unaffected by DHEA treatment, but PHA-stimulated IL-6 release was increased by 20 weeks. This enhancement of stimulated IL-6 secretion by DHEA resembles responses seen at a younger age (40), and may serve to facilitate the initiation of the acute phase reaction (42), and by stimulating B and T cell proliferation and the induction of the IL-2 receptor in mounting an immune response (38,43).

The significant increase in NK cell cytotoxicity in DHEA treated subjects was potentially related to the increased number of NK cells, both events being mediated by IL-2 stimulation. In postmenopausal women (25), an increase in NK cell cytotoxicity was also observed, but at an earlier time course of DHEA treatment, i.e., 3 weeks versus 18 weeks in men. Moreover, the inhibition of T-cell mitogenic response and the decreased number of cells expressing the CD4 antigen observed in postmenopausal women treated with DHEA (25) were not found in the present study. Although the reasons for these disparities are unknown, they suggest that a gender difference in the immune response to DHEA may exist and that sex steroids may modulate the effects of DHEA, a proposition that requires further study. The question of fluctuations in immune cell function over time also needs to be addressed. Limited data suggest a lack of variation in NK cell cytotoxicity measured over a 20-month interval (41). Because of the presence of circadian rhythm of most of the cytokines in circulation (44), such variations can clearly contribute to heterogeneity in clinical studies. It was emphasized that proper timing with uniformity of sampling would improve the usefulness of cytokine data (45).

The mechanism to account for the immune-enhancing properties of DHEA is currently unknown. The steroid may exert a direct action through binding to a specific cytosolic DHEA-binding complex (46), or by a non-genomic, direct action on immune cells. Alternatively, the effects of DHEA may be mediated by the elevation of IGF-I levels, given the observed temporal increase in bioavailable IGF-I levels and immune activation, and the demonstration of unambiguous

stimulatory effects, both in vivo and in vitro, of IGF-I on immune cells (47-49). In all probability, the activation of immune system by DHEA is not related to its androgenic potential, since androgens have a negative impact on the CD4<sup>+</sup>/CD8<sup>+</sup> ratio (50). Nonetheless, our findings add support to an emerging body of evidence suggesting that the relative levels of DHEA (immune activation) and cortisol (immune suppressor) may determine, in part, immune/metabolic function (9,51). Since ACTH and cortisol secretion remain relatively constant throughout life (52), increases and decreases in the secretion of DHEA and DHEAS, as seen during adrenarche/puberty (53) and with aging, may respectively determine, among other factors, immunocompetence. The 4-fold increase in DHEAS/cortisol ratio in response to a 50 mg dose of DHEA as seen in our age-advanced male cohort may thus be viewed as a favorable adrenal hormonal milieu for upregulating immune function, albeit at a slow pace.

In summary, in this single blind placebo-controlled trial in age-advanced men, we present preliminary evidence demonstrating that oral DHEA in a dose of 50 mg/day can significantly and safely activate the immune system by increasing the number of monocytes and B cells, stimulate T and B cell mitogenic response, increase the number of T cells expressing the IL-2R, TCR  $\gamma/\delta$  and stimulate IL-2 secretion, as well as raise circulating sIL-2R levels. In addition, DHEA treatment increased the number and cytotoxicity of NK cells and stimulated mitogen-induced IL-6 secretion without affecting its basal release. Post treatment measurements of these immune parameters were not obtained, limiting this study. While immune activation by DHEA may have potential beneficial effects in both naturally occurring and pathologic immunodeficient states, we stress that it is premature to relate these findings to clinical applications. Studies are underway in our laboratory to determine how activation of the peripheral lymphoid compartment by DHEA is translated into an ability to mount an immune response to an antigen in aging populations with special attention to define the gender differences.

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