0021-972X/00/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 85, No. 9
Printed in U.S.A.

Dehydroepiandrosterone Replacement Administration: Pharmacokinetic and Pharmacodynamic Studies in Healthy Elderly Subjects*

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ABSTRACT

Dehydroepiandrosterone (DHEA; 50 and 25 mg) and placebo tablets were orally administered daily to 24 healthy aging men and women (67.8 ± 4.3 yr) for 8 days according to a balanced incomplete block design. Nine blood tests on both the first and eighth days allowed the measurement of DHEA, its sulfate DHEAS, and metabolites: testosterone, 5α-androstan-3α,17β-diol glucuronide, estradiol, and estrone. Relatively low background levels of DHEA(S) were observed, and with the reestablishment of "young" levels, four important results were obtained. 1) Blood DHEA had an apparent terminal half-life of more than 20 h, the same order of magnitude as that of blood DHEAS, a result explainable by back-hydrolysis of the large amount of DHEAS formed after oral administration of DHEA, a mechanism providing long-lived unconjugated DHEA and metabolites. 2) The metabolic conversion of DHEAS to DHEA was significantly greater in women than in men. 3) No accumulation of steroids was observed. 4) No worrying transformation to androgen and estrogen was recorded; indeed, the limited increased estradiol in aged women could be predicted to be beneficial. These results suggested that daily oral administration of DHEA (25/50 mg) is safe in elderly subjects. The 50-mg dose was chosen for a 1 yr, double blind, placebo-controlled trial of daily oral administration of DHEA in 60- to 80-yr-old individuals (DHEAge). (*J Clin Endocrinol Metab* **85:** 3208–3217, 2000)

THE ADRENAL cortex in humans secretes dehydroepiandrosterone (DHEA, prasterone), mostly in the form of its sulfate ester (DHEAS) (1). The concentration of DHEAS in human plasma is much larger than that in any other animal; it is insignificant until approximately 7 yr of age (adrenarche), it reaches levels in the 10-μmol/L range in both sexes at 20–30 yr of age, and declines steadily in the following decades to less than 20% of the maximum after 70 yr (2–5). The physiological function of DHEA(S) (a term used to indiscriminately designate both DHEA and its sulfate) remains poorly known, even if DHEAS is by far the most abundant steroid in human plasma. No convincingly demonstrated intranuclear receptor has been described, but currently three series of findings are basic to attempting to explain DHEA(S) activity: 1) there is metabolic interconversion between DHEA and DHEAS, operated by sulfotransferase and sulfatase enzymes largely distributed in many tissues of the body (1, 6, 7); 2) the metabolic transformations of DHEA (Fig. 1) give rise to other steroidal compounds (1, 6–9), available and potentially active in peripheral tissues, such as androgens [testosterone (Testo) and 5α-dihydrotestosterone (DHT)], estrogens [estradiol (E_2) and estrone (E_1), and estrogenic Δ^5-androstene-3β,17β-diol]; and 3) neuroactive interactions of DHEA(S) with neurotransmitter receptors are involved in several behavioral functions and possibly neuroprotection (10, 11). The lack of an appropriate animal model makes study of the role of the DHEA(S) decrease in the aging process difficult (12). Studies have indicated that the decrease in concentration is mainly, if not exclusively, due to a decrease in adrenal output, initially on the order of 20–25 mg/day in young people, and not to a change in metabolism (13, 14). A number of reports concerning animals have suggested effects on metabolic parameters, immunological modulation, behavior, cancer evolution, *etc.* However, the results are difficult or impossible to extrapolate to the human situation and then to use medically; very large doses, unrelated to the physiological amounts in man, have been used, and the respective roles of both DHEA(S) itself and its hormonal metabolites, which are potentially involved, have not been studied properly. For humans, the few controlled trials of

Received January 10, 2000. Revision received May 25, 2000. Accepted June 6, 2000.

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* This work was supported by INSERM, the Région Ile-de-France, the Ministère de la Recherche, the Secrétariat d'Etat à la Santé, the AXA Insurance Co., the Fondation pour la Recherche Médicale, and the Florence Gould and Mathers Foundations. Additional financial help was obtained from Roche, the LVMH Co., the Après Association, and the Institut de Recherche Endocrinienne et Métabolique, Hôpital St. Vincent de Paul.

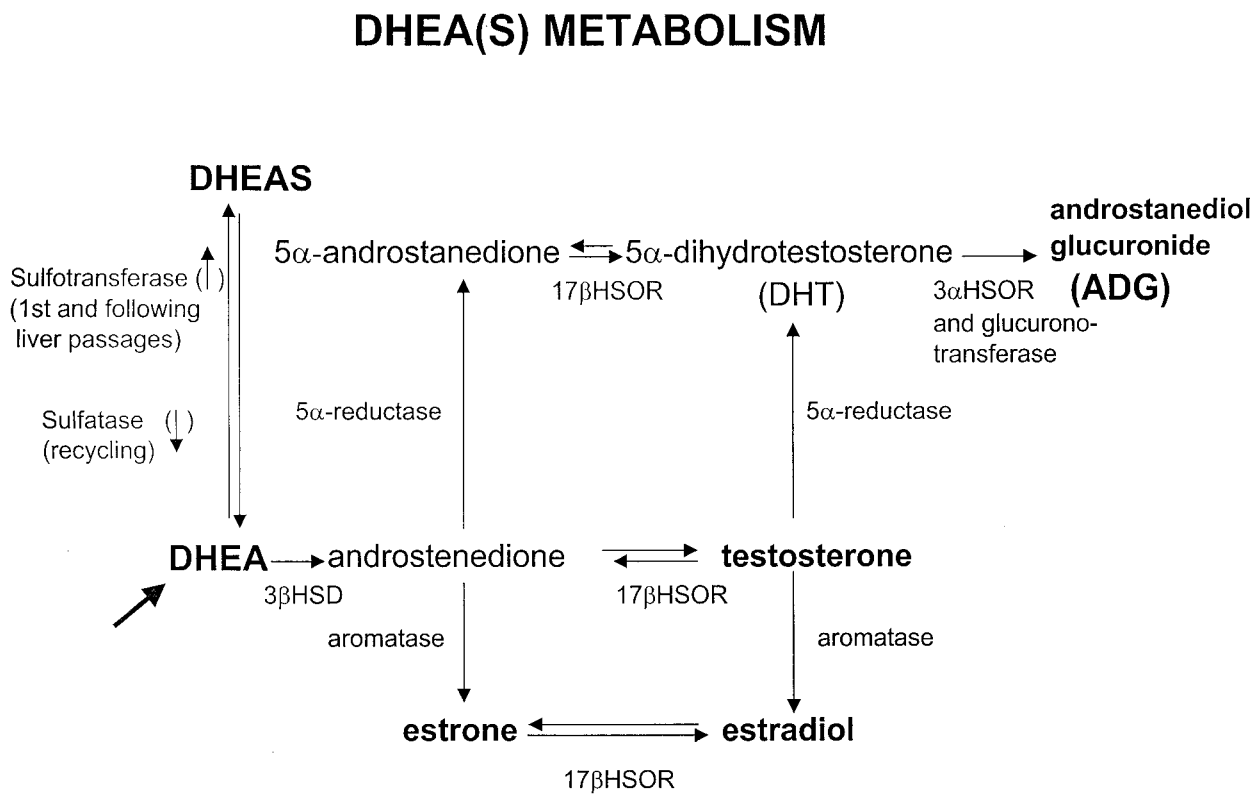

FIG. 1. DHEA(S) metabolism. Metabolic conversion of DHEA after oral administration (→). Androstanediol, 5α-Androstane-3α,17β-diol; androstenedione, Δ^4-androstene-3,17-dione; 17βH-SOR, 17β-hydroxysteroid oxydoreductase; 3αH-SOR, 3α-hydroxysteroid oxydoreductase; 3βHSD, 3β-hydroxysteroid dehydrogenase. Measured steroids are in *bold*.

DHEA administration, mostly to elderly subjects, have, however, given promising results, such as favorable immunomodulation (15, 16); increased insulin-like growth factor I levels, which also decrease in aging (17); improvement of well-being (17, 18); and, more generally, effects attributable to brain activity (reviewed in Ref. 19). However, contradictory results have been also reported for these and other parameters, such as cardiovascular protection and neurodegenerative disease, in epidemiological and clinical studies (20–23). In any case, studies of oral or percutaneous administration of DHEA with doses varying from 25–1600 mg daily have indicated that DHEA is well tolerated, and even during administration over several weeks, it provoked no or minimal deleterious effects (24, 25).

This safety has encouraged several investigators to suggest large, carefully designed and controlled trials to analyze the efficacy and tolerance of administration of DHEA(S) to the elderly to restore the plasma concentration observed in young adults. Thus, the trial DHEAge, including 280 healthy ambulatory persons, aged 60–79 yr, and lasting for 1 yr, was initiated by our group at the end of March 1998 and ended in June 1999. The first results have been published recently (26). The present study was conducted beforehand to determine the metabolism of orally administered DHEA and particularly to examine whether the proposed daily 25- or 50-mg oral doses did or did not lead to a persistent excess of DHEA(S) in the body and/or to the formation/accumulation of an excess of active hormones, such as Testo or E_2, which might have undesired effects.

Materials and Methods

Study design

A double blind, randomized, placebo cross-over study design was used. Two 8-day oral treatment periods (placebo or 25 or 50 mg DHEA given daily at 0900 h) were separated by a 2-week wash-out interval. Treatment assignment was performed according to a balanced incomplete block design. Each subject was instructed to ingest two tablets, which were both 25 mg DHEA, one DHEA and one placebo tablet, or both placebo tablets, once daily each morning at 0900 h. The two treatment periods were, for four subjects each: A, placebo then 25 mg DHEA; B, placebo then 50 mg DHEA; C, 25 mg DHEA then placebo; D, 50 mg DHEA then placebo; E, 25 mg DHEA then 50 mg DHEA; and F, 50 mg DHEA then 25 mg DHEA. As a consequence, 16 subjects (8 men and 8 women) received placebo, 16 subjects (8 men and 8 women) received 25 mg/day DHEA, and 16 subjects (8 men and 8 women) received 50 mg/day DHEA.

Subjects

Twenty-four subjects completed the study. The 12 men (age, 67.8 ± 4.3 yr) and 12 women (age, 67.9 ± 4.3 yr) had not had any health problem over the previous month. They were not obese (body mass index, 27 ± 3.7 kg/m^2 in men, 25.0 ± 3.4 kg/m^2 in women) and were not heavy smokers (<10 cigarettes/day). Illness was excluded by medical history, complete physical examination, and routine laboratory evaluation, including an electrocardiogram performed 14 days before the study. Subjects (5 women and 1 man) who took Testo or estrogen and progestin therapy were included after withdrawal of hormones for at least 1 month. Eight subjects took medication(s), for hypertension (n = 3), for hypercholesterolemia (n = 3), and for osteoarthritis (n = 3). No other drugs were allowed during the treatment period, except minor antalgics. All subjects gave their written informed consent to participate in the present experiment. The protocol was approved by the comité consul-

tatif de protection des personnes se prêtant à des recherches biomédicales (Hôpital Cochin, Paris, France). The investigation was conducted in accordance with the guidelines proposed in the Declaration of Helsinki.

Drugs

DHEA was obtained from Akzo Laboratories (Diosynth France SA, St. Denis, France). DHEA (25 mg) and placebo were manufactured by Creapharm Laboratories (Le Haillan, France) as tablets identical in appearance.

Study protocol

Each subject was hospitalized twice for 36 h each time during each of both treatment periods, starting on the day before the first and the last oral dose. Subjects arrived at the Hôpital Broussais Clinical Investigation Center at 1900 h on the evenings preceding day 1 and day 8.

On days 1 and 8 at 0800 h, after overnight fasting, an indwelling cannula was inserted into a brachial vein of each subject for blood sampling. At 0900 h, after a 1-h rest in the semirecumbent position, subjects received the first oral dose of each treatment period. They remained in the same position until 1100 h. Fluid intake was unrestricted, and subjects were given a meal 2, 6, 12, and 24 h after treatment. Blood was sampled 15 min before and immediately before drug ingestion, and 0.5, 1, 1.5, 2, 6, 12, and 24 h after drug ingestion for determinations of serum steroid levels. Potential adverse effects were recorded by means of questionnaires.

Hormone assays

Serum DHEAS was measured in serum by an automated immunoenzymatic assay, on the Serono SR1 analyzer (Serono, Milan, Italy). The correlation coefficient with standard RIA (21) was r = 0.98. The lower detection limit of the assay was 0.044 μmol/L. The intraassay coefficient of variation was 6%. The interassay coefficient of variation ranged from 3% (concentrations $<$0.27 μmol/L) to 20% (concentrations $>$2.72 μmol/L). Mean reference values in women and in men aged 60–70 yr are 2.18 and 3.70 μmol/L, respectively.

Serum DHEA, E_1, and ADG were measured by RIAs with reagents from Diagnostic Systems Laboratories, Inc. (Webster, TX). In the DHEA assay, only Adione and ADG cross-reacted significantly (0.46 and 0.24%, respectively). The intra- and interseries coefficients of variation at the level of 3.4 nmol/L were 3.1% and 4.5%, respectively. The sensitivity was 0.01 nmol/L. Reference values in women and in men, aged 60–75 yr, were 1–14 and 5–20 nmol/L, respectively. In the E_1 assay, only E_2 cross-reacted significantly (1.25%). Intra- and interseries coefficients of variation at the level of 370 pmol/L were 5.6% and 11%, respectively. References values in women and in men, aged 60–75 yr, were 22–222 and 33–240 pmol/L, respectively. In the ADG assay, only DHT glucuronide cross-reacted significantly (1.2%). The intra- and interseries coefficients of variation at levels of 0.8 and 20.0 nmol/L were 7.6% and 4.2%, and 8.1% and 4.5%, respectively. Reference values in women and in men, aged 60–75 yr, were 0.2–8.5 and 7–40 nmol/L, respectively.

Testo and E_2 were measured by RIA as previously described (27). In the Testo assay, only DHT exhibited a significant cross-reactivity (4.5%). Intra- and interseries coefficients of variation at the level of 2 nmol/L were 8% and 8.5%, respectively. Reference values in women and in men, aged 60–75 yr, were 0.17–1.7 and 8.0–35 nmol/L, respectively. In the E_2 assay, the cross-reactivity of E_1 was 0.6%. Intra- and interseries coefficients of variation at the level of 96 pmol/L were 5.8% and 2.4%, respectively. Reference values in women and in men, aged 60–75 yr, were 18–110 and 30–240 pmol/L, respectively.

Normal values for young adults, 20–40 yr old, indicated below (see *Results* and figures) were obtained by the same assays in the same laboratories.

Statistical analysis

The design was a balanced incomplete block design, and results were analyzed separately for men and women. Data for the corresponding periods were pooled because the 2-week washout interval led to patients restoring their initial hormonal status at H0 by day 1 of the second period.

We used the appropriate ANOVA method to analyze the data. When the F test was significant ($P < 0.05$), paired comparisons were performed using the Scheffe method to avoid type I error due to multiple testing. The appropriate variance estimate was taken from ANOVA for performing pairwise tests. Assumptions of ANOVA (homogeneity of variance and normality) were verified for each variable, and a natural logarithmic transformation was applied when appropriate.

As the procedure included determination of steroid levels as a function of time, we performed the above analysis on the values at 0 and 24 h and on two summarizing parameters: the maximum increase in concentration between successive determinations (C_{max}) and the area under the curve (AUC) of concentration as a function of time. AUCs were calculated using the trapezoidal rule over the 24 h, except that for plasma Testo, which was over 12 h because of the nychthemeral cycle. These kinetic values for the steroids are shown in Tables 1, 3, and 4 and Figs. 2 and 4.

Pharmacokinetic parameters were calculated using SIPHAR software (version 4.0c, Simed, Créteil, France). Data are expressed as the mean $\pm$ 1 SD in tables and the mean $\pm$ 1 SE in the graphs. $P < 0.05$ was considered statistically significant. The pharmacokinetic parameters for DHEA(S) were calculated using a model-independent approach (Table 2). The AUCs were extrapolated to infinity. At steady state (day 8), the AUC was calculated by the trapezoidal rule between a dosing interval. The elimination rate constant was estimated by log-linear regression. The maximum blood concentrations (C_{max}) and the time to C_{max} (T_{max}) were taken directly from the observed values.

In assessing pharmacokinetic parameters, it should be kept in mind that calculations were performed on day 1 over a 24-h interval according to the sampling schedule that had been designed, assuming relatively short half-lives for DHEA and DHEAS. As the measured apparent half-life for both molecules is approximately 24 h, the AUC_{0-24h} values derived from measurements on day 1 are much less (~50%) than the classical value of 80% of the individual AUC extrapolated to infinity (28).

In all groups no deviation from linearity and no pattern consistent with zero order absorption was observed. The variability was very large, but no evidence of dose dependency was found.

Results

Each C_{max} value represents the mean maximal concentration in serum steroid levels observed in the eight men or women taking the same dose of DHEA or placebo regardless of the treatment sequence. Two AUC_{0-24h} were determined for each steroid, during day 1 and day 8, respectively.

Serum DHEAS (Tables 1 and 2 and Fig. 2)

Baseline serum DHEAS levels remained unchanged with placebo. No nychthemeral cycle was noticed, similar to data observed in young adults (3).

A significant, rapid, and dose proportional increase in serum DHEAS levels was observed after oral DHEA administration. The mean maximal DHEAS concentration (C_{max}) was observed 2 h after DHEA ingestion. After 8 days of treatment with 25 or 50 mg DHEA, C_{max} reached 8.98 $\pm$ 2.7 and 13.70 $\pm$ 3.92 μmol/L, respectively ($P < 0.05$) in men and 7.70 $\pm$ 2.26 and 13.10 $\pm$ 3.80 μmol/L ($P < 0.05$) in women. Thereafter, administration of 25 mg DHEA restored serum DHEAS to levels similar to those observed in young women in the early follicular phase (1.1–7.3 μmol/L) and in young men (4.1–13.6 μmol/L). In contrast, 50 mg DHEA induced levels within the normal range for young men, whereas it increased DHEAS levels above the upper values observed in young women.

On day 8, the AUC_{0-24h} of serum DHEAS concentrations

TABLE 1. Serum DHEAS and DHEA after DHEA (50 and 25 mg/day) or placebo administration (see text)

	D1H0	D1 H24	D1C_{max}	D1AUC 24 h	D8H0	D8 H24	D8C_{max}	D8AUC 24 h
Men (mean ± 1 SD)								
DHEAS (μmol/L)				μmol/h·L				μmol/h·L
DHEA (50 mg)	2.26 ± 1.06	4.62 ± 1.22[a]	9.10 ± 1.96[a,b]	160 ± 35.3[a]	7.94 ± 3.12[a]	6.12 ± 2.23[a]	13.7 ± 3.92[a,b]	220 ± 70.8[a]
DHEA (25 mg)	2.21 ± 1.25	4.53 ± 1.06[a]	6.45 ± 1.93[a]	112 ± 29.9[a]	5.79 ± 2.34[a]	4.48 ± 1.38[a]	8.96 ± 2.72[a]	150 ± 49.0[a]
Placebo	1.39 ± 0.87	1.89 ± 1.06	2.18 ± 1.14	44 ± 24.5	2.06 ± 0.97	1.68 ± 0.82	2.28 ± 0.98	43.5 ± 19.0
F	NS	33[c]	159[c]	35[c]	32[c]	19[c]	72[c]	40[c]
DHEA (nmol/L)				nmol/h·L				nmol/h·L
DHEA (50 mg)	8 ± 3	10 ± 4	34 ± 12[a,b]	356 ± 136[a]	12 ± 3	11 ± 4[a]	32 ± 10[a,b]	399 ± 105[a,b]
DHEA (25 mg)	7 ± 4	10 ± 4	20 ± 5[a]	286 ± 109[a]	10 ± 3	10 ± 3	23 ± 7[a]	307 ± 59[a]
Placebo	7 ± 3	7 ± 3	8 ± 2	143 ± 50	7 ± 2	6 ± 2	8 ± 2	130 ± 35
F	NS	NS	39[c]	10[c]	NS	6	65[c]	46[c]
Women (mean ± 1 SD)								
DHEAS (μmol/L)				μmol/h·L				μmol/h·L
DHEA (50 mg)	1.81 ± 0.82	5.17 ± 1.39[a,b]	11.4 ± 2.70[a,b]	180 ± 41[a]	6.75 ± 1.90[a,b]	5.82 ± 1.99[a,b]	13.1 ± 3.8[a,b]	220 ± 63[a,b]
DHEA (25 mg)	1.51 ± 0.82	2.96 ± 1.06[a]	5.82 ± 1.71[a]	95 ± 30[a]	3.92 ± 1.55[a]	3.29 ± 1.28[a]	7.7 ± 2.26[a]	122 ± 41[a]
Placebo	1.23 ± 0.84	1.25 ± 0.68	1.47 ± 0.82	30 ± 19	1.22 ± 0.58	1.22 ± 0.83	1.47 ± 0.82	27 ± 16
F	NS	60[c]	82[c]	58[c]	70[c]	88[c]	121[c]	106[c]
DHEA (nmol/L)				nmol/h·L				nmol/h·L
DHEA (50 mg)	8 ± 3	15 ± 6[a]	34 ± 10[a]	443 ± 146[a]	15 ± 6[a,b]	16 ± 7	35 ± 9	496 ± 156[a]
DHEA (25 mg)	8 ± 4	13 ± 6[a]	24 ± 9[a]	336 ± 136[a]	13 ± 4	12 ± 5	30 ± 14	387 ± 134[a]
Placebo	7 ± 5	7 ± 3	9 ± 6	159 ± 96	6 ± 2	7 ± 4	8 ± 3	140 ± 65
F	NS	5	27[c]	15[c]	71[c]	5	72[c]	40[c]

F, Appropriate ANOVA. [a] When F was significant, paired comparisons were made using the Scheffe method: $P < 0.05$, D25 or D50 *vs.* placebo.
[b] $P < 0.05$, D50 *vs.* D25.
[c] $P < 0.001$; $P < 0.01$; $P < 0.05$.

TABLE 2. AUC values for DHEA and DHEAS (see text) on days 1 and 8 after DHEA (50 and 25 mg/day) or placebo administration

a) Men					b) Women				
	DHEAS		DHEA			DHEAS		DHEA	
	D1	D8	D1	D8		D1	D8	D1	D8
25 mg					25 mg				
$AUC_{0-\infty}$	234 ± 79 μmol/h·L		563 ± 184 nmol/h·L		AUC 0–∞	72 ± 29 μmol/h·L		685 ± 309 nmol/h·L	
AUC_{0-24h}		150 ± 49 μmol/h·L		307 ± 59 nmol/h·L	AUC 0–24 h		82 ± 36 μmol/h·L		712 ± 201 nmol/h·L
$t_{1/2}$ (h)	23.9 ± 6.6	23.3 ± 5.1	22.7 ± 7.2	20.1 ± 5.3	$t_{1/2}$ (h)	21.7 ± 2.5	19.4 ± 5.4	26.0 ± 8.6	23.6 ± 8.5
50 mg					50 mg				
$AUC_{0-\infty}$	348 ± 106 μmol/h·L		744 ± 306 nmol/h·L		AUC 0–∞	119 ± 33 μmol/h·L		1007 ± 562 nmol/h·L	
AUC_{0-24h}		220 ± 71 μmol/h·L		399 ± 108 nmol/h·L	AUC 0–24 h		145 ± 56 μmol/h·L		1108 ± 495 nmol/h·L
$t_{1/2}$ (h)	28.3 ± 13.7	19.4 ± 3.9	25.1 ± 10.7	18.7 ± 5.5	$t_{1/2}$ (h)	18.7 ± 2.5	19.5 ± 6.1	26.2 ± 10.7	26.6 ± 8.6

with both 25 and 50 mg doses were significantly greater than that with placebo ($P < 0.05$). In women only, AUC_{0-24h} was greater with 50 mg (220 ± 63 μmol/h·L) than with 25 mg (122 ± 41 μmol/h·L; $P < 0.05$).

In men, daily administration of 25 or 50 mg DHEA for 8 days did not cause a trend toward DHEAS accumulation, and serum DHEAS AUC_{0-24h} values on day 8 were significantly smaller than extrapolated $AUC_{0-\infty}$ values on day 1 (Table 2). The apparent half-life of DHEAS measured on day 1 and day 8 after 25 or 50 mg DHEA varied from 19.4 ± 3.9 to 28.3 ± 13.7 h.

In women, there was no accumulation of DHEAS, and the AUC at steady state (day 8) is equivalent, although smaller than that in men, to the AUC after the first dose extrapolated to infinity. As in men, there was a large intersubject variability in half-life, but mean values appeared nearly constant and independent of the dose, which further confirms the linearity of the drug disposition. The DHEAS half-life measured on day 1 and day 8 after 25 or 50 mg DHEA varied from 18.7 ± 2.5 to 21.7 ± 6.0 h.

Serum DHEA (Tables 1 and 2 and Fig. 2)

Baseline levels of serum DHEA levels were within the normal range. In men, both doses of administered DHEA increased serum DHEA levels. After 8 days with 25 or 50 mg DHEA, C_{max} and AUC_{0-24h} differed significantly from placebo ($P < 0.05$), with a dose-proportional response in men but not in women. In men and women, C_{max} with 50 mg reached 34 ± 12 and 34 ± 10 nmol/L, respectively, just above normal values observed in young men (5.2–26.0 nmol/L) and women (4.2–27.7 nmol/L; Table 1).

In men, and like DHEAS, no trend toward accumulation of DHEA on day 8 was demonstrated for either dose (Table 2). The half-life varied from 18.7 ± 5.5 to 25.1 ± 10.7 h, very similar to that of DHEAS.

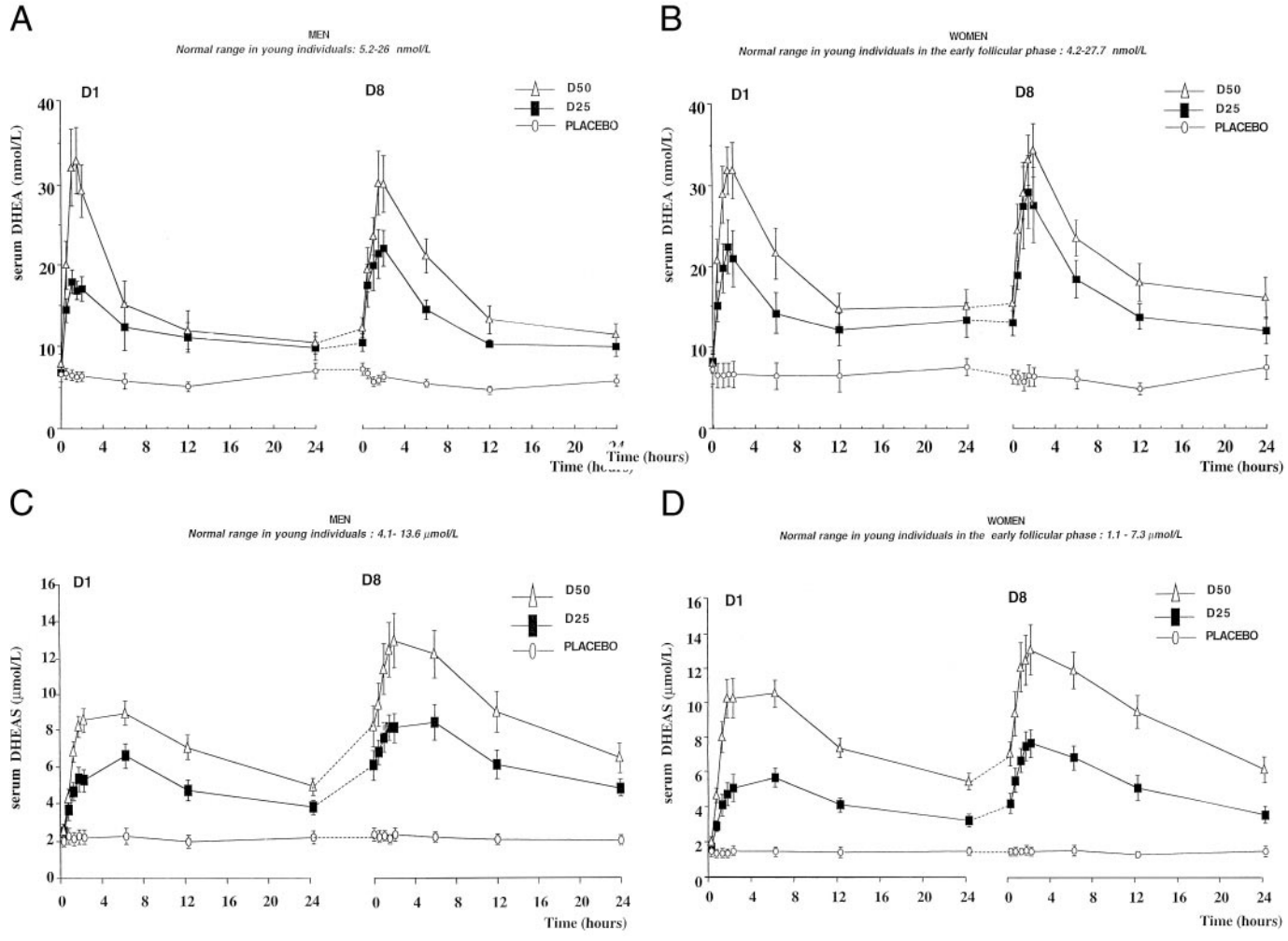

FIG. 2. Serum DHEA and DHEAS after DHEA (50 and 25 mg/day) or placebo administration.

In women, for DHEA, no trend for accumulation by day 8 was observed, and drug exposure, estimated through AUC, held a fairly constant level compared to day 1 in each dose group. The half-life varied from 23.6 ± 8.5 to 26.6 ± 8.6 h, also very similar to that of DHEAS.

Metabolites of the C_{19} steroidal series: Testo and ADG (Tables 3 and 4 and Fig. 3)

In elderly men, the serum Testo profile with placebo followed a circadian cycle similar to that observed in young men (29). In both sexes, the Testo level reached after DHEA administration was, after a modest initial increase, practically constant for the following 24 h.

In men, both 25 and 50 mg DHEA increased serum Testo nonsignificantly compared with placebo. C_{max} did not reach the normal serum levels observed in young men (11.8–34.5 nmol/L). C_{max} and AUC_{0-24h} of serum Testo *vs.* time did not differ significantly between 25 and 50 mg. In women, a significant and dose proportional increase in serum Testo levels occurred after DHEA administration for 8 days compared to placebo. With 50 mg DHEA, serum levels increased from baseline (1.0 ± 0.6 nmol/L) to peak levels (2.3 ± 1.0 nmol/L) on day 8, remaining in the normal range for young women (0.35–2.4 nmol/L). A significant and dose proportional difference in AUC_{0-24h} was observed with 25 and 50 mg DHEA compared with placebo. In fact, the increase in serum Testo levels was the same in men and women (~1 nmol/L) and was only significant for women whose baseline levels were lower.

In both sexes, baseline serum ADG levels were within the normal range for young adults. In men, after DHEA administration, serum ADG levels increased significantly, but peak values on day 8 were within the normal range observed in young men (7.5–49 nmol/L). The AUC_{0-24h} of serum ADG with DHEA differed significantly from that with placebo, but not in a dose-proportional manner. In women, after DHEA administration, serum ADG levels increased with a dose proportional effect. The AUC_{0-24h} of serum ADG significantly differed on day 8 among placebo and 25 and 50 mg DHEA. With 50 mg DHEA, serum ADG levels increased from 3 ± 2 nmol/L on day 1 to 15 ± 11 nmol/L before administration on day 8 and reached 23 ± 12 nmol/L at the peak, above the normal range for premenopausal women (1.1–10.0 nmol/L).

TABLE 3. Serum testosterone, estradiol, estrone, and androstanediol glucuronide (ADG) after DHEA (50 and 25 mg/day) or placebo administration to men

Men (mean ± 1 SD)	D1H0	D1 H24	$D1C_{max}$	D1AUC 24 h	D8H0	D8 H24	$D8C_{max}$	D8AUC 24H
				D1AUC 12 h (nmol/h·L)				D8AUC 12 h (nmol/h·L)
Testosterone (nmol/L)								
DHEA (50 mg)	15 ± 5	16 ± 5	17 ± 6	372 ± 91	16 ± 5	17 ± 4	18 ± 5	355 ± 117
DHEA (25 mg)	14 ± 2	16 ± 3	16 ± 2	361 ± 58	15 ± 2	16 ± 2	17 ± 3	350 ± 56
Placebo	13 ± 6	14 ± 6	14 ± 6	321 ± 122	14 ± 5	15 ± 5	15 ± 5	296 ± 122
F	NS	NS	NS	NS	NS	NS	NS	NS
Estradiol (pmol/L)				pmol/h·L				pmol/h·L
DHEA (50 mg)	66 ± 17	74 ± 19	87 ± 24	1786 ± 477	72 ± 19	76 ± 24	88 ± 23	1824 ± 571
DHEA (25 mg)	66 ± 15	80 ± 16	86 ± 13	1813 ± 312	66 ± 12	87 ± 15	93 ± 27	1899 ± 409
Placebo	62 ± 28	68 ± 23	79 ± 39	1558 ± 669	66 ± 28	79 ± 38	89 ± 36	1813 ± 768
F	NS	NS	NS	NS	NS	NS	NS	NS
Estrone (pmol/L)				pmol/h·L				pmol/h·L
DHEA (50 mg)	49 ± 18	73 ± 18	125 ± 26[a]	1972 ± 349[a]	82 ± 22	77 ± 13[a]	136 ± 28[a,b]	2211 ± 274[a]
DHEA (25 mg)	79 ± 41	89 ± 37[a]	120 ± 47[a]	2283 ± 1000[a]	91 ± 41	101 ± 50[a]	136 ± 82[a]	2501 ± 1291[a]
Placebo	60 ± 38	58 ± 21	77 ± 41	1311 ± 662	66 ± 28	74 ± 53	83 ± 51	1705 ± 1141
F	NS	5	11[b]	7	NS	0 5	60[c]	11[b]
Androstanediol glucuronide (nmol/L)				nmol/h·L				nmol/h·L
DHEA (50 mg)	17 ± 8	20 ± 9[a]	27 ± 10[a]	523 ± 210[a]	25 ± 13[a]	26 ± 13[a]	33 ± 15[a]	645 ± 315[a]
DHEA (25 mg)	14 ± 6	20 ± 9[a]	21 ± 9[a]	454 ± 206[a]	23 ± 12[a]	25 ± 11[a]	30 ± 15[a]	624 ± 310[a]
Placebo	10 ± 5	11 ± 6	11 ± 5	228 ± 118	11 ± 4	12 ± 5	13 ± 5	261 ± 115
F	NS	6	7	7	23[c]	19[c]	39[c]	36[c]

F, Appropriate ANOVA.
[a] When F was significant, paired comparisons were made using the Scheffe method: $P < 0.05$, D25 or D50 *vs.* placebo and $P < 0.05$, D50 *vs.* D25.
[b] $P < 0.01$.
[c] $P < 0.001$.

TABLE 4. Serum testosterone, estradiol, estrone, and androstanediol glucuronide (ADG) after DHEA (50 and 25 mg/day) or placebo administration to women

Women (mean ± 1 SD)	D1H0	D1 H24	$D1C_{max}$	D1AUC 24 h	D8H0	D8 H24	$D8C_{max}$	D8AUC 24H
				D1AUC 12 h (nmol/h·L)				D8AUC 12 h (nmol/h·L)
Testosterone (nmol/L)								
DHEA (50 mg)	1.03 ± 0.58	1.47 ± 0.59[a]	1.97 ± 0.86[a,b]	36 ± 15[a,b]	1.64 ± 0.67[a,b]	1.7 ± 0.6[a,b]	2.31 ± 0.95[a,b]	44 ± 17[a,b]
DHEA (25 mg)	0.72 ± 0.24[a]	1.14 ± 0.33[a]	1.19 ± 0.41[a]	23 ± 8[a]	1.25 ± 0.39[a]	1.15 ± 0.3[a]	1.66 ± 0.44[a]	29 ± 7[a]
Placebo	1.04 ± 0.64	1.03 ± 0.60	1.06 ± 0.63	21 ± 14	0.94 ± 0.54	1.07 ± 0.5	1.06 ± 0.59	20 ± 11
F	6	19[c]	54[c]	30[c]	52[c]	34[c]	63[c]	44[c]
Estradiol (pmol/L)				pmol/h·L				pmol/h·L
DHEA (50 mg)	22 ± 5	27 ± 6	36 ± 10	706 ± 192[a,b]	29 ± 5[a,b]	31 ± 9[a]	42 ± 10[a]	822 ± 226[a]
DHEA (25 mg)	22 ± 5	24 ± 5	34 ± 5	619 ± 90	26 ± 6	29 ± 4	43 ± 11[a]	790 ± 184[a]
Placebo	23 ± 7	20 ± 4	28 ± 7	548 ± 129	22 ± 6	22 ± 5	27 ± 8	522 ± 96
F	NS	NS	NS	5	8[d]	4	6	8[d]
Estrone (pmol/L)				pmol/h·L				pmol/h·L
DHEA (50 mg)	39 ± 23.2	68 ± 25	107 ± 38[a,b]	1920 ± 770[a,b]	76 ± 29[a]	83 ± 22[a,b]	134 ± 33[a,b]	2297 ± 592[a]
DHEA (25 mg)	39 ± 15.7	50 ± 13	76 ± 12[a]	1310 ± 441[a]	63 ± 24[a]	63 ± 20[a]	107 ± 22[a]	1794 ± 498[a]
Placebo	42 ± 20.0	40 ± 21	55 ± 20	978 ± 544	42 ± 21	40 ± 20	53 ± 21	945 ± 541
F	NS	16[c]	32[c]	41[c]	9[d]	19[c]	36[c]	19[c]
Androstanediol glucuronide (nmol/L)				nmol/h·L				nmol/h·L
DHEA (50 mg)	3 ± 2	11 ± 9[a,b]	17 ± 12[a,d]	281 ± 223[a,b]	15 ± 11[a,b]	17 ± 11[a,b]	23 ± 12[a,b]	428 ± 276[a,b]
DHEA (25 mg)	3 ± 2	5 ± 3[d]	7 ± 4[a]	129 ± 77[a]	6 ± 3[a]	8 ± 4[a]	11 ± 5[a]	199 ± 91[a]
Placebo	3 ± 1	2 ± 1	3 ± 2	56 ± 34	3 ± 2	3 ± 2	3 ± 2	59 ± 32
F	NS	23[c]	64[c]	63[c]	31[c]	40[c]	144[c]	105[c]

F, Appropriate ANOVA.
[a] When F was significant, paired comparisons were made using the Scheffe method: $P < 0.05$, D25 or D50 *vs.* placebo.
[b] $P < 0.05$, D50 *vs.* D25.
[c] $P < 0.001$.
[d] $P < 0.01$.

Serum estrogens (Tables 3 and 4 and Fig. 4)

In men, serum E_2 levels did not change after placebo or DHEA treatment, whereas serum E_1 concentrations increased dramatically after DHEA administration. Peak values and changes in serum E_1 *vs.* time significantly differed compared with placebo values. C_{max}, but not AUC $_{0-24h}$,

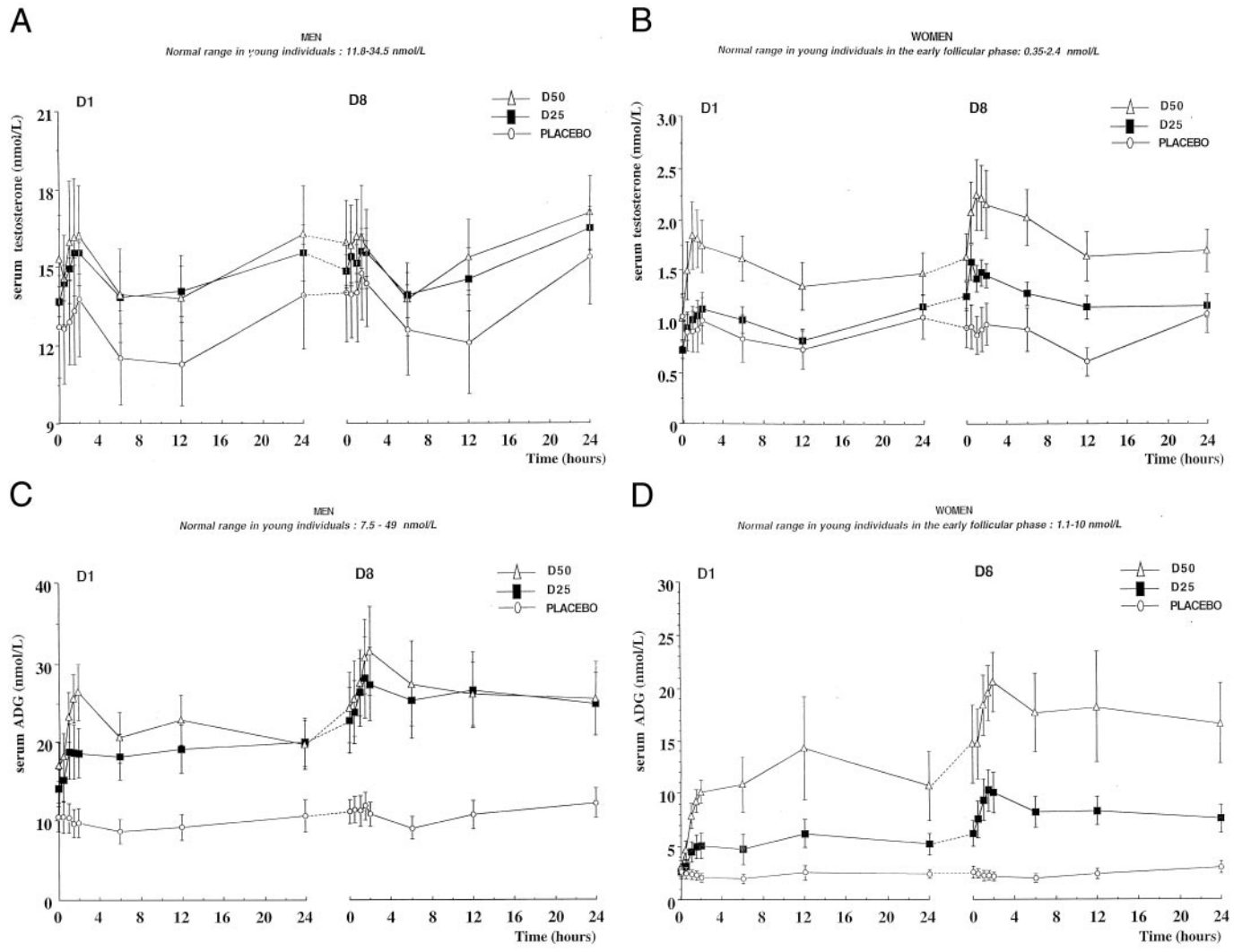

FIG. 3. Serum Testo and ADG after DHEA (50 and 25 mg/day) or placebo administration.

differed significantly according to the dose. Peak values of E_2 and E_1 were within the normal levels range for young men (36.7–147 and 55–333 pmol/L, respectively).

In women, E_2 peak values and AUC_{0-24h} increased significantly compared with placebo values after 8 days with 25 or 50 mg DHEA. Peak levels, reaching 40 pmol/L, were less than the normal levels in young women during the early follicular phase (70–345 pmol/L). No difference in C_{max} or AUC related to DHEA dose was observed. For E_1, a significant increase was observed with DHEA treatment compared with placebo, within the normal values (37–333 pmol/L). C_{max}, but not AUC_{0-24h} differed significantly according to the dose.

Discussion

The present study is of interest for aged individuals who may benefit from a replacement administration of DHEA to prevent some of the deleterious effects of aging. Two doses were selected for preliminary pharmacokinetic and pharmacodynamic studies before implementing a randomized controlled clinical trial for 1 yr. A double blind, randomized, placebo cross-over study design was thus conducted, as described above.

DHEAS-DHEA

At baseline, serum DHEAS levels were significantly higher in men than in women (~130%). This difference has been reported by numerous researchers (3, 5, 30). No gender difference was demonstrated for DHEA levels at baseline.

After DHEA administration, in both sexes there was a rapid and dose proportional increase in DHEAS levels. In men, 25 or 50 mg DHEA administration restored levels to the normal range for young adults. In women, 50 mg DHEA administration induced peak levels just above the normal levels observed in young adults on day 8. No accumulation was observed in either sex after administration for 8 days.

The apparent half-life of DHEAS was about 24 h, longer than that determined after iv administration of radioactive DHEAS tracer to young adults (1, 13). It has been reported that an increase in DHEA(S), whether secreted or administered, tends to increase its MCR and/or its volume of distribution (30–32). Fifty milligrams of DHEA is a high daily

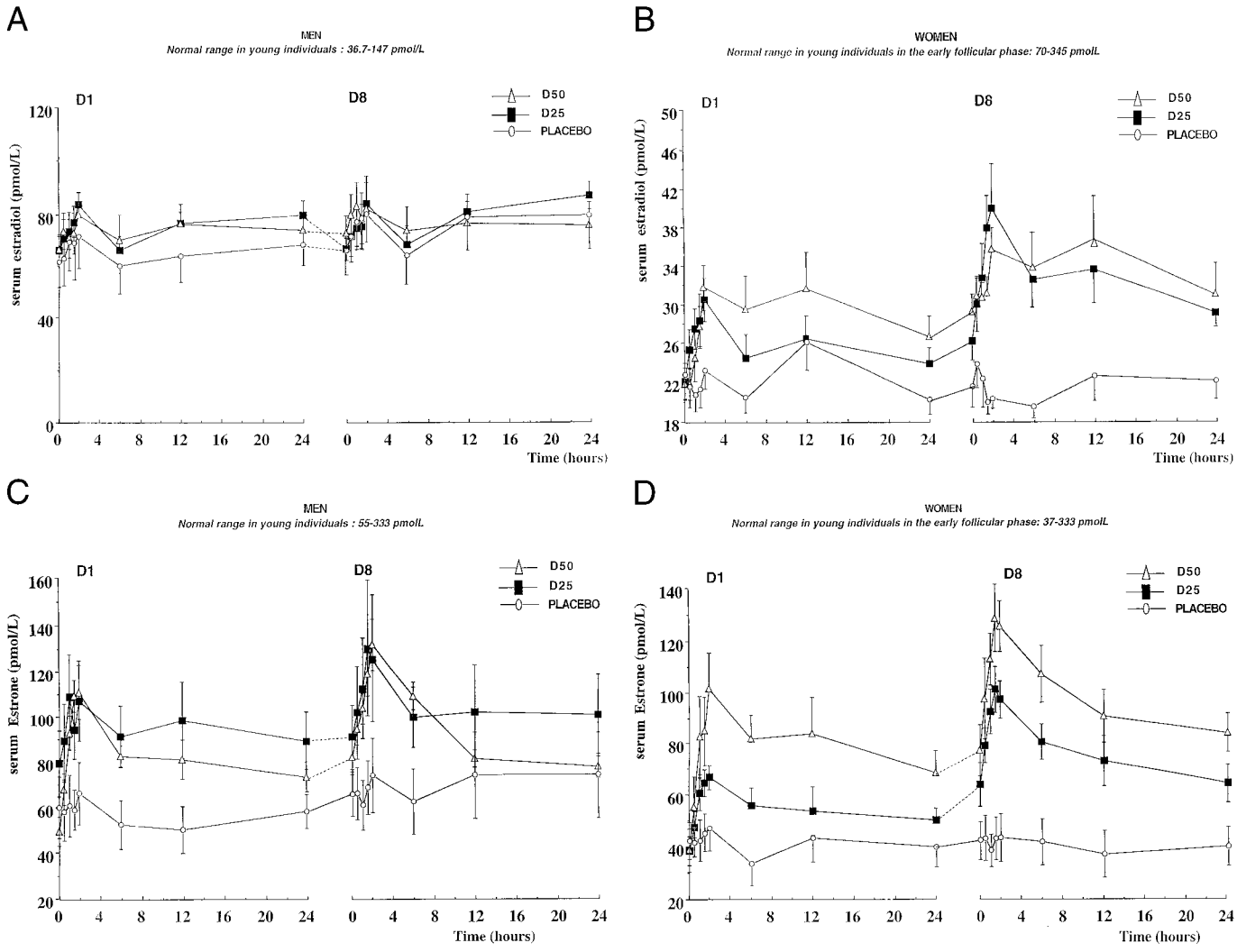

FIG. 4. Serum E_2 and E_1 after DHEA (50 and 25 mg/day) or placebo administration.

dose compared to the adrenal physiological secretion observed in young adults (~25 mg daily). After ingestion, DHEA is mostly sulfated in liver. Most DHEAS is bound to plasma albumin, but no specific high affinity transport protein has been described. Whether an entero-hepatic cycle is involved is unknown (33, 34). No data concerning the age-related modifications of DHEA(S) distribution are available (13). The MCR seems to be unaltered with aging. The mechanisms underlying this long apparent half-life of DHEAS have to be further studied.

DHEA levels also showed a notable significant increase. In both sexes, levels reached were just above normal values observed in young adults. As described in *Materials and Methods*, our free DHEA assay is highly specific and cannot be significantly contaminated by DHEAS and other steroids. Technically, we carefully tried to avoid any contamination of the DHEA levels by artifactual release of the unconjugated steroid from the large concentrations of DHEAS (12). Therefore, the endogenous DHEA levels measured should represent the sum of DHEA taken orally and having escaped liver and gut sulfation, plus DHEA originating from the sulfatase hydrolysis of DHEAS formed. The latter is by far the most important quantitatively. This very likely explains a most striking result of the present study: the apparent half-life of DHEA was much longer than expected in both men and women and was similar to the DHEAS half-life. This suggests a role for the rate-limited production from long-lived DHEAS and therefore a so called flip-flop phenomenon, *i.e.* the apparent half-life mainly reflects the metabolic back-conversion of DHEAS to DHEA. As no iv data with the same amount of DHEA are available, a definite conclusion is not possible; however, experiments with iv administration of radioactive DHEAS strongly suggest the preponderant role of this metabolic back-conversion to DHEA. It had been previously reported that the DHEA half-life and MCR, as measured from isotopic steroid iv experiments, were about 30 min and 2000 L/h, respectively (1, 13, 35, 36), similar to those of other hormonal steroids.

Our hypothesis is that oral administration of DHEA leads to the synthesis of the limiting step for DHEA availability. Our results are consistent with studies of the pharmacokinetics and peripheral conversion of DHEA to androgens and

estrogens in young healthy females after dexamethasone suppression, where it was demonstrated that 50 mg was the suitable dose for DHEA replacement for adrenal insufficiency (18, 37). After an oral dose, the half-life of DHEA is much longer than that previously described in tracer iv studies (1, 13, 35, 36). In the rat (admittedly very different from the human in steroid metabolism), it was observed that the bioavailability of DHEA was greater after percutaneous than oral administration, avoiding the hepatic first pass effect (38). From our data, oral administration to humans should be preferred to obtain an as high as possible long-lived DHEA level, derived from metabolically formed DHEAS.

No accumulation of DHEA(S) was demonstrated after this daily administration of a physiological dose of DHEA (39). These results are reassuring in terms of safety and led us to choose the 50-mg DHEA dosage for the 1-yr clinical trial to try to maximize the exposure to DHEA(S) without risk. The same conclusion has been reached when S. Tummala and F. Svec analyzed published data of serum levels after DHEA administration (unpublished results).

Sex differences

The DHEA(S) results differed greatly according to gender. On day 8 of DHEA administration, whatever the dose, the DHEAS AUC_{0-24h} values were greater in men than in women. Clearly, after either daily dose of administered DHEA, DHEA blood levels were persistently higher in women than in men (although DHEAS levels remained higher in men than in women). The DHEA AUC_{0-24h} values in women were about twice those in men. The relatively higher exposure to DHEA observed in women might be related to the higher dose they received per kg BW. However, the results may indicate an intrinsic sex difference in steroid metabolism; the metabolic conversion of DHEAS to DHEA appears more effective in women. From a pharmacokinetic point of view, this would suggest that the optimal dose to be administered to women in a chronic regimen could be smaller than that for men; indeed, such a deduction is consistent with the clinical results of the DHEAge trial (26). Several reports have previously outlined that DHEA(S) metabolism differs between men and women (34, 36, 40–42). This difference may be of importance when considering the peripheral effects of DHEA metabolites *vs.* the impact on nervous system DHEAS, as discussed previously (12).

Hormonal metabolites

In both sexes, whatever the DHEA dose, peak levels of E_2 and Testo remained within the normal range for young adults. These results are also relevant to the safety of DHEA administration. Interestingly, the increase in E_2 peak levels after administration of 50 mg DHEA are compatible with some estrogenic action, especially for the prevention of bone loss in postmenopausal women. E_2 levels about 23 pmol/L at baseline reached 42 pmol/L on day 8. A specific study of the effect of DHEA(S) on bone loss and risk of fractures would be of interest, as several researchers have demonstrated the role of low doses of estrogen in both sexes (43–45), and the limited transformation of DHEA(S) into active hormones such as E_2 may be beneficial as replacement therapy and safe for prolonged administration.

ADG levels are considered a reliable marker of the total pool of Testo and may indicate Testo production otherwise undetectable from blood levels (via an intra/paracrine system) (8), but possibly active if the steroid formed interacts locally with an androgen receptor. These ADG levels reflect the increase in the 17β-hydroxyl pathway of Testo metabolism in tissues (46) better than the increase in serum Testo levels, and therefore, Testo is not the only index of potential androgenic activity. ADG levels observed after DHEA administration vary greatly in both sexes. In women, ADG levels increased in a dose proportional manner to reach supraphysiological levels, even with large SDS; clinically, the 1-yr trial has not produced evidence of deleterious androgenic symptoms. In any case, ADG may not be a perfectly reliable index of androgenic activity; the formation of Testo and its degradation to ADG may occur in cells or tissues devoid of androgen receptor, and in addition, ADG may derive from DHEAS without intermediary conversion to Testo (47). Therefore, ADG is more an index of androgen consumption than of androgenic activity.

Conclusion

From all these considerations, the present report suggest that, in healthy 60–79 year old subjects, 50 mg DHEA is a safe and potentially effective dose. But these results may apply only to elderly subjects with relatively low DHEAS levels at baseline, as compared to young individuals; whether the same 50 mg DHEA administration earlier in life would give excessive steroid concentration cannot be known from this study. Globally the results of the one-year trial implemented in Assistance Publique - Hôpitaux de Paris (DHEAge) (26) are consistent with the data reported in this paper.

Acknowledgments

We thank Prof. Joël Ménard, Director of the Centre d'Investigations Clinique, Hôpital Broussais (Paris, France), Prof. James F. Tait (Hants, United Kingdom), and Prof. Christopher Longcope (University of Massachusetts, Boston, MA) for their precious advice and help. We thank the members of the Centre d'Investigation Clinique INSERM-AP-HP, Hôpital Broussais and Hôpital Necker, the Fondation Nationale de Gérontologie, the Institut de Recherche Endocrinienne et Métabolique, Dr. K. Rajkowski for editorial help, and Stéphanie Delhaye, Marilyn Ourtou, and Claudine Lévy for their skilled secretariat assistance.

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