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Six-month oral dehydroepiandrosterone supplementation in early and late postmenopause

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ABSTRACT

The adrenal production of the Δ5-androgens, dehydroepiandrosterone (DHEA) and its sulfate ester dehydroepiandrosterone sulfate (DHEAS), declines linearly with aging. The evidence that DHEA or DHEAS administration may alleviate some of the problems related to aging has opened new perspectives for clinical research. The present study aims to investigate the effects of a 6-month DHEA supplementation in early and late postmenopausal women, with normal or overweight body mass index (BMI), on the level of circulating steroids, sex hormone binding globulin (SHBG), β-endorphin and gonadotropins, and on the adrenal gland response to dexamethasone suppression and adrenocorticotropic hormone (ACTH) stimulation.

Early postmenopausal women (50–55 years) both normal weight (BMI 20–24, n = 9) and overweight (BMI 26–30, n = 9) and late postmenopausal women (60–65 years) both of normal weight and overweight, were treated with oral DHEA (50 mg/day). Circulating DHEA, DHEAS, 17-OH pregnenolone, progesterone, 17-OH progesterone, allopregnanolone, androstenedione, testosterone, dihydrotestosterone, estrone, estradiol, SHBG, cortisol, luteinizing hormone, follicle stimulating hormone and β-endorphin levels were evaluated monthly and a Kupperman score was performed. The product/precursor ratios of adrenal steroid levels were used to assess the relative activities of the adrenal cortex enzymes. Before and after 3 and 6 months of therapy, each women underwent an ACTH stimulating test (10 μg i.v. in bolus) after dexamethasone administration (0.5 mg p.o.) to evaluate the response of cortisol, DHEA, DHEAS, androstenedione, 17-OH pregnenolone, allopregnanolone, progesterone and 17-OH progesterone.

The between-group differences observed before treatment disappeared during DHEA administration. Levels of 17-OH pregnenolone remained constant during the 6 months. Levels of DHEA, DHEAS, androstenedione, testosterone and dihydrotestosterone increased progressively from the first month of treatment. Levels of estradiol and estrone significantly increased after the first/second month of treatment. Levels of SHBG significantly decreased from the second month of treatment only in overweight late postmenopausal women, while the other

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groups showed constant levels. Progesterone levels remained constant in all groups, while 17-OH progesterone levels showed a slight but significant increase in all groups. Allopregnanolone and plasma β-endorphin levels increased progressively and significantly in the four groups, reaching values three times higher than baseline. Levels of cortisol and gonadotropins progressively decreased in all groups.

The product/precursor ratios of adrenal steroid levels at the sixth month were used to assess the relative activities of the adrenal cortex enzymes and were compared to those found before therapy. The 17,20-desmolase, sulfatase and/or sulfotransferase, 17,20-lyase and 5α-reductase activities significantly increased, while the 3β-hydroxysteroid-oxidoreductase activity did not vary. On the contrary, the 11-hydroxylase and/or 21-hydroxylase activities showed a significant decrease after 6 months of treatment.

In basal conditions, dexamethasone significantly suppressed all the adrenal steroids and this suppression was greater after 3 and 6 months of treatment for DHEA, DHEAS and allopregnanolone, while it remained unchanged for other steroids. Before treatment, ACTH stimulus induced a significant response in all parameters; after the treatment, it prompted a greater response in Δ5- and Δ4-androgens, progesterone and 17-OH progesterone, while cortisol responded less in both younger and older normal-weight women. The endometrial thickness did not show significant modifications in any of the groups of postmenopausal women during the 6 months of treatment. Treatment with DHEA was associated with a progressive improvement of the Kupperman score in all groups, with major effects on the vasomotor symptoms in the early postmenopausal women.

In conclusion, the present findings confirm that DHEA supplementation produces physiological and supraphysiological modifications in steroid milieu and adrenal function. The beneficial effects of DHEA on the quality of life and in reverting the aging process may be related to changes in the release of adrenal products and/or peripheral steroids, with an increase in anxiolytic (allopregnanolone), anabolic (androstenedione, testosterone, dihydrotestosterone) and estrogenic (estrone, estradiol) molecules, a beneficial decrease in cortisol and increase in pituitary β-endorphin production.

INTRODUCTION

Dehydroepiandrosterone (DHEA) and its sulfate ester, dehydroepiandrosterone sulfate (DHEAS) have been proposed as a 'fountain of youth'[1]. In humans, the synthesis and release of DHEA and DHEAS (DHEA(S)) in adrenal reticularis zone cells declines linearly with age, from the third decade of life, when it reaches its peak, to less than 20% at the age of 70. In contrast, cortisol circulating levels do not follow this trend and it is responsible for the corticotropin-releasing factor (CRF)–adrenocorticotropic hormone (ACTH)–cortisol feedback loop, which regulates adrenal steroid secretion. We still do not know which factors are involved in regulating the mithosis of DHEA-secreting cells, while it is clear that ACTH is the main stimulus for secretion[2–4].

In the young adult, the half-life ($t_{1/2}$) of DHEA is 15–30 min, its metabolic clearance is 1700 l/day and its blood production rates about 2–7 mg/day. The $t_{1/2}$ of DHEAS is 10 h, and its metabolic clearance rate is about 15 l/day, with a blood production rate of 25–30 mg/day. As a consequence, circulating DHEAS is maintained at a constant level during a 24-hour period. On the other hand, DHEA levels show nyctohemeral variations with an amplitude of 50%, with lower levels in the late afternoon. After a single exogenous 50 mg DHEA administration, serum DHEA concentrations increase, peaking after 2.5 ± 2.1 h, and its $t_{1/2}$ is 8.9 ± 3.6 h[5].

Some authors have reported an overlap of values between sexes, showing lower DHEA(S) levels (10–25%) in women than in men, with a parallel age-related decrease in both sexes. In postmenopausal women, DHEA ultradian and circadian rhythms are markedly attenuated. This suggests a reduction in 17,20-desmolase activity, which determines a further decline in Δ5-androgen production[6].

Other factors which may modify circulating levels of DHEA(S) are body mass index (BMI) and insulin/glucose balance. Obesity has been shown to be associated with an increased adrenal activity in both adults and children. In fact, in obese prepubertal and pubertal girls, circulating Δ5-androgen levels are significantly higher than in those of normal-weight age-matched girls[7,8]. Some studies have not reported any link between DHEA(S) levels and BMI in adult women in either premenopause or postmenopause[9] while other trials have shown a reduction or an increase in circulating Δ5-androgen levels in obese subjects[10–14].

The consistent evidence that DHEA(S) administration improves some of the problems related to aging has opened new perspectives for clinical research[15–18]. In fact, low-dose DHEA or DHEAS administration (50–100 mg/day) in postmenopausal women induces improvement in psychological and physical well-being, ameliorates the immune system function and bone mineral density[19–24] with no steroid accumulation during the treatment[25]. Indeed, DHEA administration increases the level of sex hormones (DHEAS, testosterone and estradiol) to those of young adults, and improves libido, with no modification of the cardiovascular parameters and no side-effects[25].

In a recent study, we investigated the endocrine, neuroendocrine and behavioral effects of a 3-month DHEAS supplementation (50 mg/day) in early postmenopausal women, showing a significant increase in circulating basal androgens (DHEA(S), androstenedione and testosterone) and estrogens (estradiol and estrone) to values similar to those of fertile women[26]. Moreover, DHEAS produced an increase in plasma β-endorphin levels and, similarly to estradiol, restored the β-endorphin response to specific adrenergic, serotoninergic and opiatergic neuroendocrine stimuli and improved subjective vasomotor and psychological symptoms[26].

Since no conclusive data on DHEA treatment in early and late postmenopausal subjects are available, we aimed to investigate the effects of a 6-month DHEA supplementation (50 mg/day) in early and late postmenopausal women, with normal or overweight BMI, on the circulating steroids, sex hormone binding globulin (SHBG), β-endorphin, gonadotropins and on the adrenal gland response to dexamethasone suppression and ACTH stimulation.

MATERIALS AND METHODS

Subjects

A group of 31 healthy postmenopausal women (age range 50–65 years) was studied. The protocol was approved by the local ethical committee of the University of Pisa and informed consent was obtained from each woman before beginning the study. The study was performed to evaluate the effect of oral DHEA administration in early postmenopausal women (50–55 years) both of normal weight (BMI = 20–24) (group A, $n = 9$, BMI = 22.1 ± 0.5) and overweight (BMI = 26–30) (group B, $n = 9$, BMI = 28.2 ± 0.5), and late postmenopausal women (60–65 years) both normal weight (group C, $n = 7$, BMI = 22.5 ± 0.6) and overweight (group D, $n = 6$, BMI = 27.9 ± 0.4). The trial was prospective and lasted 6 months. Among the women enrolled in the study, only six were mild smokers (< 8 cigarettes/day); the others were non-smokers.

All women were given DHEA (50 mg p.o./day) (Rottapharm, Milan, Italy) for 6 months, between 8.00 and 9.00 am, except for the days on which the ACTH test was performed. The exclusion criteria were previous or current estrogen-dependent neoplasia, thromboembolic disease, liver, pancreatic or renal disease, and diabetes mellitus or any other endocrine disease.

Protocol

Prior to the study, breast and gynecological examinations, including pelvic ultrasound examination and mammography, were performed. A transvaginal ultrasound examination was performed before treatment and after 3 and 6 months of treatment to evaluate the endometrial thickness.

The women were evaluated monthly throughout the trial period. A blood specimen to measure baseline levels of circulating DHEA, DHEAS, 17-OH pregnenolone, progesterone, 17-OH progesterone, allopregnanolone, androstenedione, testosterone, dihydrotestosterone, estrone, estradiol, SHBG, cortisol, luteinizing hormone (LH), follicle stimulating hormone (FSH) and β-endorphin was drawn at 8.00 am, after overnight fasting and before any drug administration, at each visit. The specimens were centrifuged and plasma was stored at −20°C until assay.

Dexamethasone suppression and ACTH test

Before therapy, and after 3 and 6 months of therapy, each woman underwent an ACTH-stimulating test between 8.00 and 8.30 am, after overnight fasting. All women were studied after dexamethasone (0.5 mg p.o.) at 11.00 pm on the day before, and 24 hours after the last dose of DHEA. An indwelling i.v. catheter was placed in the antecubital vein for blood sampling. An ACTH

test (10 μg i.v. in bolus) was performed to evaluate the response of cortisol, DHEA, DHEAS, androstenedione, 17-OH pregnenolone, allopregnanolone, progesterone and 17-OH progesterone.

Blood specimens were collected before ACTH injection and at 15, 30, 60, 90, 120 and 150 minutes following injection. Specimens were centrifuged and serum was stored at −20°C until assay.

Subjective symptoms

Before therapy, and after 1, 2 and 6 months of therapy, a Kupperman questionnaire was completed. It included complaints such as subjective vasomotor and psychological symptoms. The use of the Kupperman score has been described elsewhere[27]. Each symptom appears on a rating scale that has a range from 0 to 3. The lower end of the scale for each symptom is described as 'none', while the higher is 'marked'. Each patient was following by the same gynecologist throughout the study.

Assays

For each subject all hormones were measured during the same assay. Serum concentrations of DHEA, DHEAS, androstenedione, testosterone, 17-OH progesterone, cortisol, 17-OH pregnenolone, estrone, estradiol, SHBG, LH and FSH were measured by specific commercially available radioimmunoassay (RIA) kits (Radim®; Pomezia, Rome, Italy). The sensitivities of the assays for DHEA and DHEAS were 0.2 ng/ml and 0.02 μg/ml respectively; the intra- and interassay coefficients of variation (CVs) were 3.8 and 6.9 for DHEA and 4.0 and 8.5 for DHEAS. The sensitivities of the assays for androstenedione and testosterone were 0.03 ng/ml and 0.017 ng/ml, respectively, the intra- and interassay CVs were 4.3 and 6.0 for androstenedione and 5.1 and 7.8 for testosterone. The 17-OH progesterone assay had a sensitivity of 0.01 ng/ml and the intra- and interassay CVs were 5.2 and 6.3. For cortisol and 17-OH pregnenolone the sensitivities were 0.9 μg/l and 0.1 ng/ml respectively, and the intra- and interassay CVs were 3.6 and 6.2 for cortisol and 4.5 and 6.1 for 17-OH pregnenolone. For the estradiol and estrone the sensitivities were 10 and 1.2 pg/ml respectively, and the intra- and interassay CVs were 2.1 and 3.5 for estradiol and 4.4 and 6 for estrone. The sensitivities of the assays for LH and FSH were 0.20 mIU/ml and 0.18 mIU/ml respectively, and the intra- and interassay CVs were 2.8 and 3.3 for LH and 1.97 and 4.11 for FSH.

Allopregnanolone assay

Allopregnanolone evaluation was performed after ether extraction and chromatographic partition on Sep-Pak C18 cartridges (Waters Co., Milford, USA) using a specific previously described RIA method[28]. The sensitivity of the assay was 10 pg/tube and the intra- and interassay CVs were 7.2 and 9.1, respectively.

Beta-endorphin assay

Each plasma sample was submitted to extraction and chromatographic partition using Sep-Pak C_{18} cartridges, and the β-endorphin evaluation was performed using a specific previously described RIA method[29].

The sensitivity of the β-endorphin radioimmunoassay was 2.5 pg/ml and the intra- and interassay CVs were 6 and 9, respectively.

Adrenal enzymatic activity assessment

Before treatment, and after 6 months of treatment the product/precursor ratios in basal samples were calculated to assess the relative activities of the adrenal enzymes. Products and precursors, and the enzymes assayed by their ratios, were as follows: (DHEA/17-OH pregnenolone: 17,20-desmolase; DHEAS/DHEA: sulfatase and/or sulfotransferase; allopregnanolone/progesterone: 5α reductase; androstenedione: 3β-OH-dehydrogenase; cortisol/ 17-OH progesterone: 11- and/or 21-hydroxylase; androstenedione/17-OH progesterone: 17,20-lyase).

Statistics

The obtained data were expressed as mean ± SEM. Basal hormone levels, product/precursor ratios of adrenal steroids and endometrial thickness evaluation were analysed with a multiple analysis of

variance (ANOVA) and compared by using a paired Student's *t* test.

The effects of the dexamethasone suppression were examined by using the percentage of decrease with respect to basal values and the data were analysed by ANOVA. The area under the curve (AUC) (subtracted from the basal value) was used to evaluate the response to the ACTH tests.

RESULTS

Clinical findings

During treatment, no changes in body weight, uterine bleeding, abnormal event or side-effects, were recorded on diary cards by the patients. Ultrasound evaluation of the endometrial thickness did not show significant modifications within any of the groups after 6 months of therapy (Table 1). In all treatment groups the Kupperman score showed a progressive improvement. In particular, in early postmenopausal women (groups A and B) all items ameliorated with respect to subjective vasomotor instability and psychological disturbances (Table 2). In basal conditions, late postmenopausal subjects (groups C and D) did not show relevant vasomotor symptoms, but during treatment the items regarding psychological disturbances significantly improved (Table 2).

Biochemical evaluation

Basal conditions

Circulating steroids, SHBG, β-endorphin and gonadotropin levels (Table 3) Levels of DHEA and DHEAS showed a significant age-related decrease only in women of normal weight (groups A and C). Androstenedione levels were higher in younger (groups A and B) than in older postmenopausal women (groups C and D). Estrone levels were higher in the two groups of overweight women (groups B and D), showing highest levels in group B. Among the late postmenopausal women, the overweight group (D) showed significantly lower 17-OH progesterone and allopregnanolone levels than the other women (groups A, B and C). Cortisol levels were lower in both groups of overweight women (groups B and D). Levels of SHBG were significantly higher in late postmenopausal women (groups C and D) with respect to early postmenopausal women (groups A and B). Levels of LH were significantly lower in the older overweight women (group D), while no significant between-group differences were observed in basal 17-OH pregnenolone, testosterone, dihydrotestosterone, estradiol, progesterone, β-endorphin and FSH levels.

Adrenal enzymatic activity (Figure 1) The product/precursor ratios of baseline adrenal steroid levels were used to assess the relative activities of the adrenal cortex enzymes (shown in brackets). The DHEA/17-OH pregnenolone (17,20-desmolase) ratio was significantly higher in groups A and B and the DHEAS/DHEA ratio (sulfatase and/or sulfotransferase) was higher in group A. The androstenedione/DHEA (3β-hydroxysteroid-oxidoreductase) ratio was significantly higher in group A, while the androstenedione/17-OH progesterone (17,20-lyase), the allopregnanolone/progesterone (5α-reductase) and the cortisol/17-OH progesterone (11- and/or 21-hydroxylase) ratios did not show any difference among the studied groups.

Effects of dexamethasone suppression (Table 4) In basal conditions, dexamethasone significantly suppressed all adrenal steroids; the inhibition rate ranged from 83 to 90% for cortisol and from 25 to

Table 1 Endometrial thickness (mm) in normal-weight and overweight early postmenopausal women (groups A and B) and in normal-weight and overweight late postmenopausal women (groups C and D). Values are given as mean ± SEM

	Treatment group			
Time point	*Group A*	*Group B*	*Group C*	*Group D*
Before treatment	2.7 ± 0.3	2.8 ± 0.4	2.1 ± 0.3	3.1 ± 0.5
After 3 months of treatment	2.2 ± 0.2	2.0 ± 0.4	1.7 ± 0.1	2.6 ± 0.2
After 6 months of treatment	2.3 ± 0.3	2.5 ± 0.3	2.0 ± 0.4	2.9 ± 0.4

Table 2 Kupperman score (mean ± SEM) for vasomotor symptoms, psychological symptoms and total score in normal-weight and overweight early postmenopausal women (groups A and B) and in normal-weight and overweight late postmenopausal women (groups C and D)

	Kupperman score		
	Vasomotor symptoms	*Psychological symptoms*	*Total score*
Group A			
Before treatment	18.8 ± 3.2	8.8 ± 1.8	27.6 ± 4.6
After 1 month of treatment	11.6 ± 3.2*	5.9 ± 1.5*	18.4 ± 2.6*
After 2 months of treatment	8.0 ± 2.5**	2.7 ± 0.6**	11.4 ± 2.3*
After 3 months of treatment	6.7 ± 1.9**	2.1 ± 0.7**	9.3 ± 2.3**
After 6 months of treatment	4.3 ± 1.7**	2.0 ± 0.6**	6.8 ± 1.6**
Group B			
Before treatment	18.0 ± 3.8	8.2 ± 1.7	26.2 ± 5.0
After 1 month of treatment	13.1 ± 3.3*	7.2 ± 1.3	20.2 ± 4.2*
After 2 months of treatment	9.9 ± 2.9**	5.3 ± 1.1*	15.0 ± 3.9*
After 3 months of treatment	7.2 ± 2.3**	3.4 ± 0.9*	10.4 ± 3.1**
After 6 months of treatment	4.7 ± 1.3**	2.8 ± 0.6**	7.2 ± 1.5**
Group C			
Before treatment	2.0 ± 1.05	8.2 ± 1.6	10.4 ± 1.9
After 1 month of treatment	0.5 ± 0.5 (NS)	5.2 ± 1.5	5.7 ± 11.2*
After 2 months of treatment	0.5 ± 0.5 (NS)	3.8 ± 0.9*	4.2 ± 0.8**
After 3 months of treatment	0.5 ± 0.5 (NS)	3.5 ± 0.4**	3.9 ± 0.4**
After 6 months of treatment	0.5 ± 0.5 (NS)	2.0 ± 0.9**	2.5 ± 0.2**
Group D			
Before treatment	2.7 ± 1.2	12.7 ± 1.7	17.7 ± 2.2
After 1 month of treatment	1.5 ± 0.5 (NS)	10.7 ± 2.1	12.7 ± 1.1*
After 2 months of treatment	0.5 ± 0.5 (NS)	7.0 ± 1.7*	8.5 ± 1.4**
After 3 months of treatment	0.5 ± 0.5 (NS)	6.2 ± 1.6**	6.6 ± 1.2**
After 6 months of treatment	0.5 ± 0.5 (NS)	2.1 ± 0.8**	2.7 ± 0.5**

*$p < 0.05$ vs. before treatment; **$p < 0.001$ vs. before treatment; NS, not significant vs. before treatment

41% for allopregnanolone. In particular, the percentage of decrease after dexamethasone was significantly higher for DHEA in group A, and for DHEAS and progesterone in groups A and C. No significant differences with regard to age and body weight were observed in terms of 17-OH pregnanolone, androstenedione, 17-OH progesterone, allopregnanolone and cortisol after dexamethasone suppression.

Adrenocorticotropic hormone test (Table 5) The ACTH stimulus performed at 8.00 am, after dexamethasone suppression, induced a significant response in all parameters, showing a pattern as reported for example in Group A in Figure 2. The areas under the curves were calculated in order to evaluate the differences among the groups. The 17-OH pregnenolone response was similar in group A, B and D, while it was significantly lower in group C compared to group A. The DHEA response was significantly higher in group A, while DHEAS responded more in group C. The progesterone response was higher in group D than in the other groups. The responses of androstenedione, 17-OH progesterone, allopregnanolone and cortisol to ACTH were similar among the groups.

Effects of DHEA supplementation

Levels of 17-OH pregnenolone, DHEA and DHEAS (Figure 3) In each group, 17-OH pregnenolone levels remained constant during the 6 months of therapy. DHEA levels (range 3.0 ± 0.2 (group C)–3.9 ± 0.2 ng/ml (group A)) increased significantly and progressively from the first month of treatment, reaching highest levels at the fifth or sixth month with values ranging from 12.8 ± 0.7 (group C) to 14.2 ± 0.8 ng/ml (group D)

Table 3 Basal endocrine evaluation in normal-weight and overweight early postmenopausal women (groups A and B) and in normal-weight and overweight late postmenopausal women (groups C and D)

	Treatment group			
	Group A	*Group B*	*Group C*	*Group D*
17-OH pregnenolone (ng/ml)	0.44 ± 0.04	0.40 ± 0.06	0.66 ± 0.1	0.42 ± 0.06
DHEA (ng/ml)	3.91 ± 0.2	3.88 ± 0.3	3.00 ± 0.2†	3.29 ± 0.3
DHEAS (μg/ml)	0.77 ± 0.05	0.55 ± 0.07	0.46 ± 0.13†	0.48 ± 0.07
Androstenedione (ng/ml)	1.61 ± 0.2*	1.52 ± 0.2*	0.6 ± 0.1	0.86 ± 0.1
Testosterone (ng/ml)	0.53 ± 0.04	0.44 ± 0.06	0.41 ± 0.07	0.53 ± 0.03
Dihydrotestosterone (pg/ml)	50.2 ± 7.3	44.0 ± 6.2	40.5 ± 6.8	42.0 ± 5.6
Estrone (pg/ml)	41.3 ± 3.2	59.6 ± 2.3**	34.6 ± 2.1	45.3 ± 2.8††
Estradiol (pg/ml)	18.6 ± 1.3	19.7 ± 2.4	16.0 ± 1.5	17.5 ± 1.6
Progesterone (ng/ml)	0.33 ± 0.05	0.32 ± 0.07	0.31 ± 0.07	0.34 ± 0.07
17-OH progesterone (ng/ml)	0.90 ± 0.07	0.78 ± 0.07	0.88 ± 0.11	0.50 ± 0.07†††
Allopregnanolone (pg/ml)	176.0 ± 18.5	162.0 ± 24.2	158.8 ± 20.7	147.5 ± 21.9†††
Cortisol (μg/l)	201.2 ± 6.8	141.2 ± 10**	210.6 ± 7.9	180.7 ± 13**
β-endorphin (pg/ml)	17.0 ± 3.1	21.1 ± 1.0	14.7 ± 1.7	16.3 ± 1.5
SHBG (ng/ml)	8.3 ± 0.7	7.2 ± 0.7	11.2 ± 2.4***	11.3 ± 3.0***
LH (mIU/ml)	34.8 ± 3.1	34.3 ± 3.0	32.4 ± 3.5	24.3 ± 3.5†††
FSH (mIU/ml)	64.9 ± 4.5	63.8 ± 5.4	71.8 ± 6.8	51.8 ± 9.9

DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; FSH, follicle stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone binding globulin; †$p < 0.05$ vs. group A; ††$p < 0.05$ vs. group C; †††$p < 0.05$ vs. groups A, B and C; *$p < 0.05$ vs. groups C and D; **$p < 0.05$ vs. groups A and C; ***$p < 0.05$ vs. groups A and B

(Figure 3c,d). DHEAS levels (range 0.46 ± 0.13 (group C)–0.77 ± 0.05 μg/ml (group A) increased significantly and progressively during the first 3–4 months, with more than a two-fold increase at the fourth and fifth months, reaching values ranging from 2.4 ± 0.3 (group D) to 2.8 ± 0.4 μg/ml (group A) (Figure 3e,f). No between-group significant differences in circulating DHEA and DHEAS were observed throughout the trial period among the four groups.

Levels of androstenedione, testosterone and dihydrotestosterone (Figure 4) Androstenedione levels (range 0.6 ± 0.1 (group C)–1.6 ± 0.2 ng/ml (group A)) increased significantly and progressively during the first 3–4 months, reaching a three–four-fold increase at the fifth and sixth months (3.9 ± 0.2 (group D)–4.7 ± 0.6 ng/ml (group A)). No differences were observed in circulating androstenedione levels in the four groups (Figure 4a,b). Testosterone levels (range 0.41 ± 0.07 (group C)–0.53 ± 0.04 ng/ml (group A)) significantly increased during the first 3 months of treatment, with a four-fold increase at the fifth and sixth months, ranging from 1.5 ± 0.1 (group B) to 2.2 ± 1.2 ng/ml (group D). Among the late postmenopausal women, the overweight group (group D) showed significantly higher levels from the third month of treatment than the other women (Figure 4c,d). Dihydrotestosterone levels (range 40.5 ± 6.8 (group C)–50.2 ± 7.3 pg/ml (group A)) increased significantly and progressively during the first 3–4 months of treatment, reaching highest levels at the sixth month (200.2 ± 11.4 (group D)–320.5 ± 18.5 pg/ml (group C)). No differences were found between early and late overweight postmenopausal women. However, normal-weight late postmenopausal women showed higher dihydrotestosterone levels from the fourth month of treatment (Figure 4e,f).

Levels of estrone, estradiol and SHBG (Figure 5) In all groups, estrone levels (range 34.6 ± 2.1 (group C)–59.6 ± 2.3 pg/ml (group B)) significantly increased from the second month, reaching a three–four-fold increase at the fifth month (110.4 ± 7.2 (group A)–155.2 ± 22.2 pg/ml

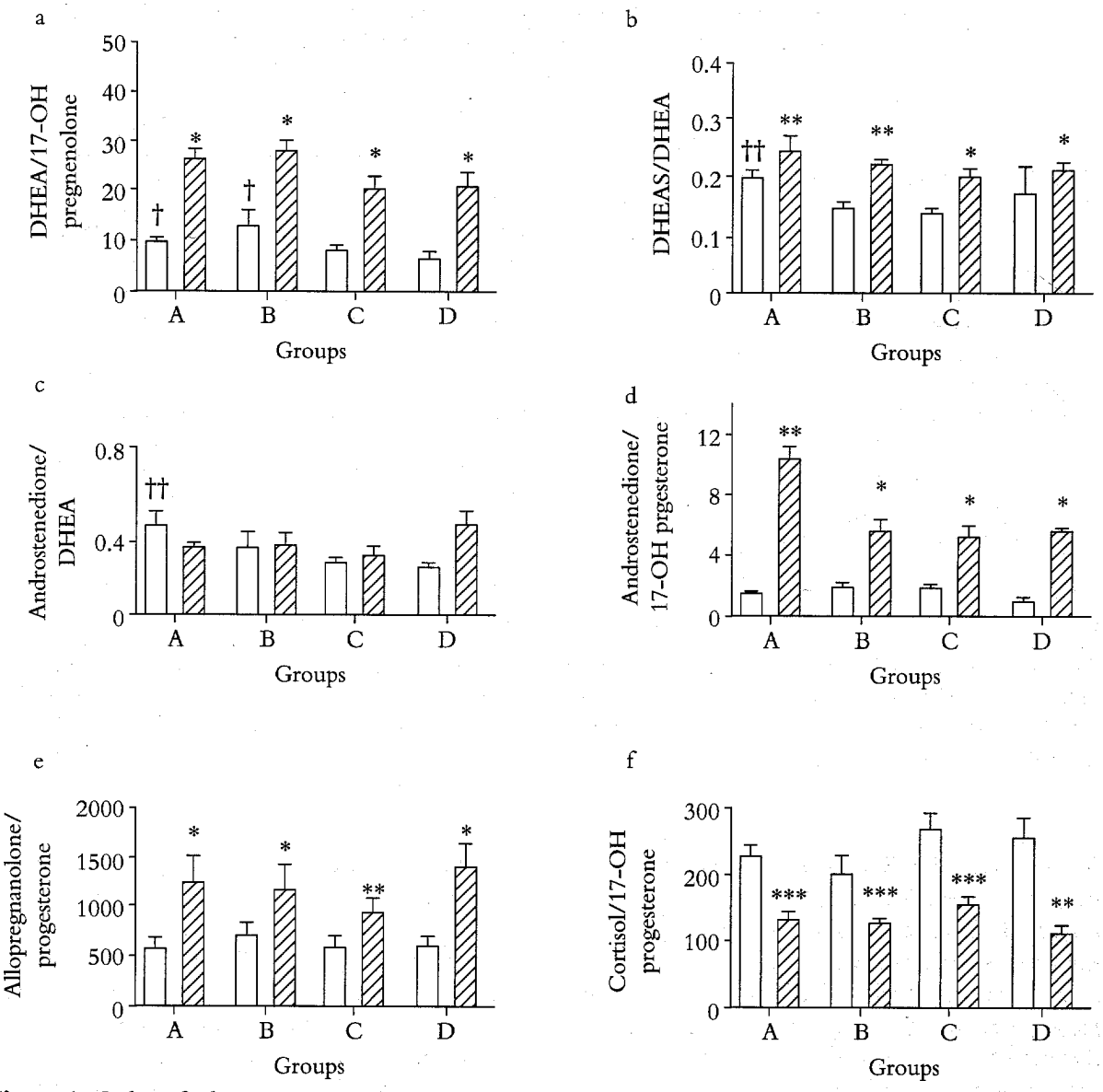

Figure 1 Index of relative activities of steroidogenetic enzymes of the adrenal cortex before and after 6 months' DHEA supplementation in normal-weight and overweight early postmenopausal women (groups A and B) and normal-weight and overweight late postmenopausal women (groups C and D). Relative activity indexes are ratios of serum levels of steroid pairs adjacent in the appropriate biosynthetic pathway. (a) 17,20-Desmolase (DHEA (dehydroepiandrosterone)/17-OH pregnenolone ratio); (b) sulfatase and/or sulfotransferase (DHEAS (dehydroepiandrosterone sulfate/DHEA ratio); (c) 3β-hydroxysteroid-oxidoreductase (androstenedione/DHEA ratio); (d) 17,20-lyase (androstenedione/17-OH progesterone ratio); (e) 5α-reductase (allopregnanolone/ progesterone ratio); (f) 11- and/or 21-hydroxylase (cortisol/17-OH progesterone ratio). Values are shown as mean ± SEM. Results were analyzed to detect statistically significant differences between groups before DHEA treatment ($^{\dagger}p < 0.05$ vs. groups C and D; $^{\dagger\dagger}p < 0.05$ vs. groups B, C and D) and within groups before and after DHEA treatment ($^{*}p < 0.05$ vs. basal ratio; $^{**}p < 0.01$ vs. basal ratio; $^{***}p < 0.001$ vs. basal ratio). ▭, before DHEA treatment; ▨, after 6 months of DHEA treatment

(group D)) (Figure 5a,b). The late postmenopausal women showed higher levels of estrone than the younger subjects from the third month (Figures 1a and b). A significant and progressive rise in estradiol levels (range 16.0 ± 1.5 (group C)–18.6 ± 1.3 pg/ml (group B)) was observed in all groups of patients from the first month, reaching values four to five times higher at the fifth and sixth months (76.4 ± 5.8 (group C)–98.2 ± 10.6 pg/ml (group D)) (Figure 5c,d). The highest values were

Table 4 Percentage decrease in levels of tested compounds after dexamethasone suppression in normal-weight and overweight early postmenopausal women (groups A and B) and normal-weight and overweight late postmenopausal women (groups C and D). Results were analyzed to detect statistically significant differences between groups before DHEA treatment ($^{\dagger}p < 0.05$ vs. groups C and D; $^{\dagger\dagger}p < 0.01$ vs. groups B and D) and within groups before and after DHEA treatment ($*p < 0.05$ vs. before treatment; $**p < 0.001$ vs. before treatment). Values are given as mean ± SEM

	17-OH Pregnenolone	*DHEA*	*DHEAS*	*Andro-stenedione*	*Progesterone*	*17-OH Progesterone*	*Allo-pregnanolone*	*Cortisol*
Group A								
Before	−49.4 ± 6.2	−63.4 ± 3.4†	−61.4 ± 3.8††	−61.3 ± 4.1	−50.2 ± 3.2††	−53.4 ± 3.5	−25.1 ± 3.2	−87.2 ± 1.2
After 3 months	−42.4 ± 4.3	−69.4 ± 6.2*	−66.4 ± 1.2	−63.1 ± 5.1	−42.9 ± 5.4	−59.5 ± 3.4	−48.1 ± 3.2*	−88.7 ± 0.9
After 6 months	−47.8 ± 6.4	−74.7 ± 2.8**	−68.3 ± 1.3*	−60.9 ± 2.6	−45.3 ± 7.2	−59.6 ± 4.0	−52.9 ± 3.2**	−89.8 ± 0.6
Group B								
Before	−51.5 ± 5.9	−55.3 ± 4.0	−41.3 ± 5.9	−55.4 ± 3.8	38.4 ± 1.9	−54.4 ± 4.2	−38.7 ± 3.2	−83.1 ± 3.1
After 3 months	−55.6 ± 5.0	−70.8 ± 2.1**	−67.1 ± 4.4*	−43.4 ± 6.8	−44.9 ± 5.8	−57.3 ± 2.5	−52.6 ± 3.2*	−86.6 ± 2.2
After 6 months	−48.5 ± 3.7	−72.4 ± 1.6**	−64.6 ± 7.4*	−69.2 ± 6.7	−45.5 ± 5.4	−48.7 ± 5.2	−56.2 ± 3.2**	−85.5 ± 3.2
Group C								
Before	−44.7 ± 5.7	−52.4 ± 4.7	−60.2 ± 6.4††	−54.1 ± 2.0	−57.7 ± 3.4††	−53.7 ± 3.2	−35.6 ± 3.2	−90.8 ± 0.8
After 3 months	−45.0 ± 4.5	−70.1 ± 3.5**	−59.2 ± 6.7	−70.5 ± 3.9**	−65.2 ± 4.9	−57.6 ± 2.3	−54.8 ± 3.2**	−89.8 ± 0.7
After 6 months	−42.3 ± 6.7	−72.9 ± 2.8**	−67.1 ± 4.1*	−73.7 ± 2.5**	−57.9 ± 5.9	−51.5 ± 3.6	−54.9 ± 3.2**	−89.4 ± 0.8
Group D								
Before	−39.2 ± 5.0	−53.3 ± 2.7	−48.9 ± 7.5	−58.3 ± 2.9	−34.5 ± 4.7	-51.2 ± 3.5	−41.2 ± 3.2	−90.8 ± 2.0
After 3 months	−44.3 ± 4.3	−62.7 ± 1.6*	−55.4 ± 6.6	−60.9 ± 3.6	−35.7 ± 5.8	−56.8 ± 8.4	−48.6 ± 3.2*	−87.6 ± 1.3
After 6 months	−44.3 ± 3.1	−58.7 ± 3.8*	−66.7 ± 5.8*	−56.9 ± 4.1	−40.6 ± 5.7	−55.6 ± 4.8	−47.8 ± 3.2*	−85.1 ± 2.3

DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate

reached in group D patients. Levels of SHBG (range 7.2 ± 0.4 (group B)–11.3 ± 2.1 ng/ml (group D)) significantly decreased from the second month of treatment only in postmenopausal overweight subjects (8.3 ± 1.2 ng/ml (group D)), while the other groups showed constant levels (Figure 5e,f).

Levels of progesterone, 17-OH progesterone, allopregnanolone (Figure 6) Progesterone levels remained constant in all groups under treatment (Figure 6a,b). Levels of 17-OH progesterone (range 0.50 ± 0.07 (group D)–0.90 ± 0.07 ng/ml (group A)) showed a slight but significant increase in all groups from the first or second month of therapy reaching highest values at the fifth month (1.1 ± 0.07 ng/ml (group A)) (Figure 6c,d). Group D maintained significantly lower 17-OH progesterone levels throughout the trial period. Allopregnanolone levels (range 147.5 ± 21.9 (group D)–176.0 ± 18.5 pg/ml (group A)) progressively and significantly increased in the four groups and tripled at the fourth and fifth month (470.2 ± 40.7 (group D)–485.9 ± 38.5 pg/ml (group C)). There were no significant between-group differences in allopregnanolone levels throughout the study (Figure 6e,f).

Levels of cortisol, β-endorphin, LH and FSH (Figure 7) Cortisol levels (range 141.2 ± 10 (group B)–210.6 ± 7.9 μg/l (group C)) progressively decreased in all groups, reaching the lowest values at the 8 sixth month (111.3 ± 7.4 (group B)–148.7 ±.1 μg/l (group A)). The decrease appeared to be significant from the second month of therapy in the older women (groups C and D) and from the third month in the two groups of younger women (groups A and B) (Figure 7a,b). Conversely, plasma β-endorphin levels (range 14.7 ± 1.7 (group C)–21.1 ± 1.0 pg/ml (group B)) began to rise from the first month in each group of patients and tripled at the sixth month (64.5 ± 6.8 (group C)–72.1 ± 8.5 pg/ml (group D)) (Figure 7c,d). During the treatment, in older subjects, plasma β-endorphin levels were significantly higher in overweight than in normal-weight age-matched subjects. Levels of

Table 5 Mean ± SEM area under the curve (AUC) in response to ACTH test in normal-weight and overweight early postmenopausal women (groups A and B) and in normal-weight and overweight late postmenopausal women (groups C and D). Statistical analysis: between groups under basal conditions ($^{\dagger}p < 0.05$ vs. group C; $^{\dagger\dagger}p < 0.05$ vs. groups B, C and D; $^{\dagger\dagger\dagger}p < 0.05$ vs. groups A and B; $^{\dagger\dagger\dagger\dagger}p < 0.05$ vs. groups A, B and C); within groups before and after DHEA treatment ($^{*}p < 0.05$ vs. before; $^{**}p < 0.001$ vs. before)

	17-OH Pregnenolone	*DHEA*	*DHEAS*	*Andro-stenedione*	*Progesterone*	*17-OH Progesterone*	*Allo-pregnanolone*	*Cortisol*
Group A								
Before	109.2 ± 16.4†	1793.7 ± 144.9††	101.5 ± 14.6	232.8 ± 60.9	54.6 ± 4.1	114.9 ± 27.9	25788.7 ± 7916.1	29457.7 ± 1960.4
After 3 months	144.5 ± 20.4	1813.2 ± 113.2	195.1 ± 39.8*	393.4 ± 61.5*	78.8 ± 4.7*	89.8 ± 8.3	27451.9 ± 4237.5	26179.8 ± 1916.7
After 6 months	145.1 ± 26.5	2111.6 ± 138.7*	196.2 ± 22.4*	465.3 ± 83.7*	72.1 ± 4.7*	102.9 ± 6.7	28899.7 ± 5659.6	21413.9 ± 1486.2**
Group B								
Before	91.7 ± 12.7	1346.7 ± 187.8	87.0 ± 8.8	135.1 ± 30.0	57.7 ± 7.6	110.8 ± 22.3	16492.0 ± 3490.4	25796.0 ± 1393.8
After 3 months	146.7 ± 27.6	1579.5 ± 235.3	120.1 ± 36.8	249.8 ± 28.3*	80.0 ± 7.4*	189.3 ± 79.9	19043.3 ± 2034.2	23934.6 ± 1148.8
After 6 months	151.6 ± 22.9*	1540.9 ± 180.0	182.5 ± 38.8*	378.1 ± 43.7**	87.7 ± 9.8**	127.5 ± 22.8	25800.9 ± 3417.3*	21799.9 ± 609.8**
Group C								
Before	74.5 ± 4.9	1385.9 ± 169.8	170.4 ± 23.9†††	233.8 ± 35.7	58.0 ± 6.7	183.4 ± 37.9	17351.2 ± 3058.2	29728.0 ± 1634.2
After 3 months	102.8 ± 12.1	1323.2 ± 135.9	210.7 ± 23.3	308.9 ± 38.5*	97.8 ± 7.6**	132.9 ± 49.2	29109.6 ± 4857.3*	26526.1 ± 1687.8*
After 6 months	93.3 ± 13.2	1508.0 ± 122.7	192.3 ± 21.3	346.9 ± 54.1**	100.6 ± 6.3**	261.8 ± 57.7*	22293.1 ± 2071.9	24732.5 ± 2196.8*
Group D								
Before	96.9 ± 17.7	1078.7 ± 155.4	137.9 ± 32.1	204.5 ± 32.3	78.9 ± 5.3††††	91.4 ± 8.7	17163.7 ± 1879.7	25760.2 ± 1245.1
After 3 months	96.3 ± 8.8	1055.7 ± 186.7	169.9 ± 23.9	324.8 ± 42.5*	86.4 ± 6.8	193.0 ± 41.3*	18567.5 ± 1679.1	25930.2 ± 2329.9
After 6 months	97.6 ± 13.4	1311.6 ± 131.3*	171.1 ± 12.5	355.5 ± 44.7**	107.7 ± 8.3*	196.4 ± 33.9*	16366.2 ± 3239.9	27328.1 ± 3327.4

DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate

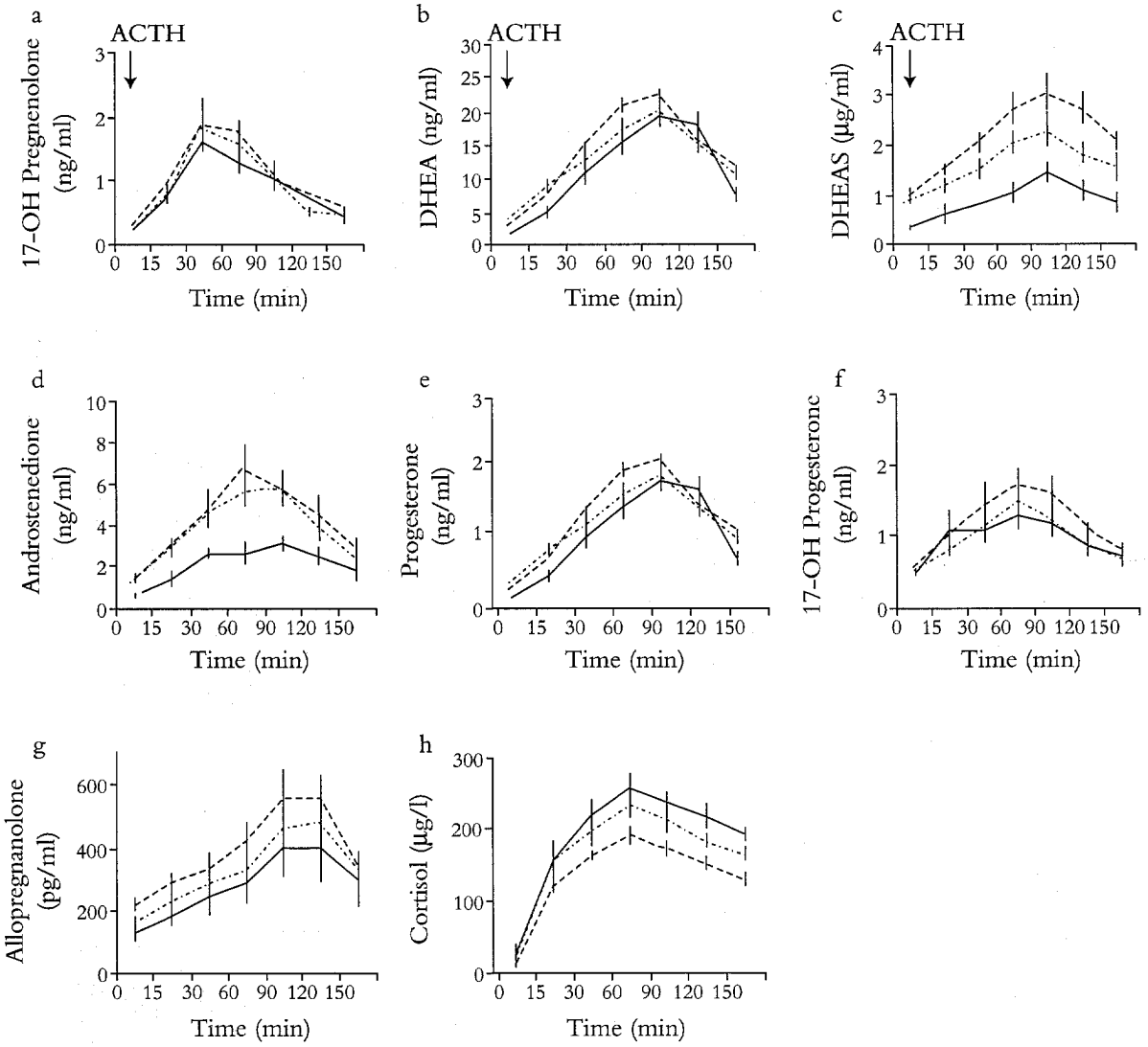

Figure 2 Serum levels of (a) 17-OH pregnenolone; (b) dehydroepiandrosterone (DHEA); (c) dehydroepiandrosterone sulfate (DHEAS); (d) androstenedione; (e) progesterone; (f) 17-OH progesterone; (g) allopregnanolone; and (h) cortisol in response to adrenocorticotropic hormone (ACTH) stimulation after 12 h dexamethasone suppression. Values are shown as mean ± SEM. ———, Before DHEA treatment; -·-·-, after 3 months of DHEA treatment; -----, after 6 months of DHEA treatment

LH (range 24.3 ± 3.5 (group D)–34.8 ± 3.1 mIU/ml (group A)) decreased in all four groups, reaching the lowest values at the sixth month (11.8 ± 2.8 (group D)–26.3 ± 2.7 mIU/ml (group B)). The decrease was significant from the second month in the older subjects (groups C and D) and from the third month in the younger subjects (groups A and B) (Figure 7e,f). Levels of FSH (51.8 ± 9.9 (group D)–71.8 ± 6.8 mIU/ml (group C)) progressively and significantly decreased from the second/third month in the four groups, reaching values ranging from 34.1 ± 9.7 (group D) to 38.2 ± 4.9 mIU/ml (group C)) (Figure 7g,h).

Adrenal enzymatic activity (Figure 7) The product/precursor ratios of adrenal steroid levels at the sixth month of therapy were used to assess the relative activities of the adrenal cortex enzymes and were compared to those found before therapy. DHEA/17-OH pregnenolone (17,20-desmolase activity), DHEAS/DHEA (sulfatase and/or sulfotransferase activity), androstenedione/17-OH progesterone (17,20-lyase activity) and

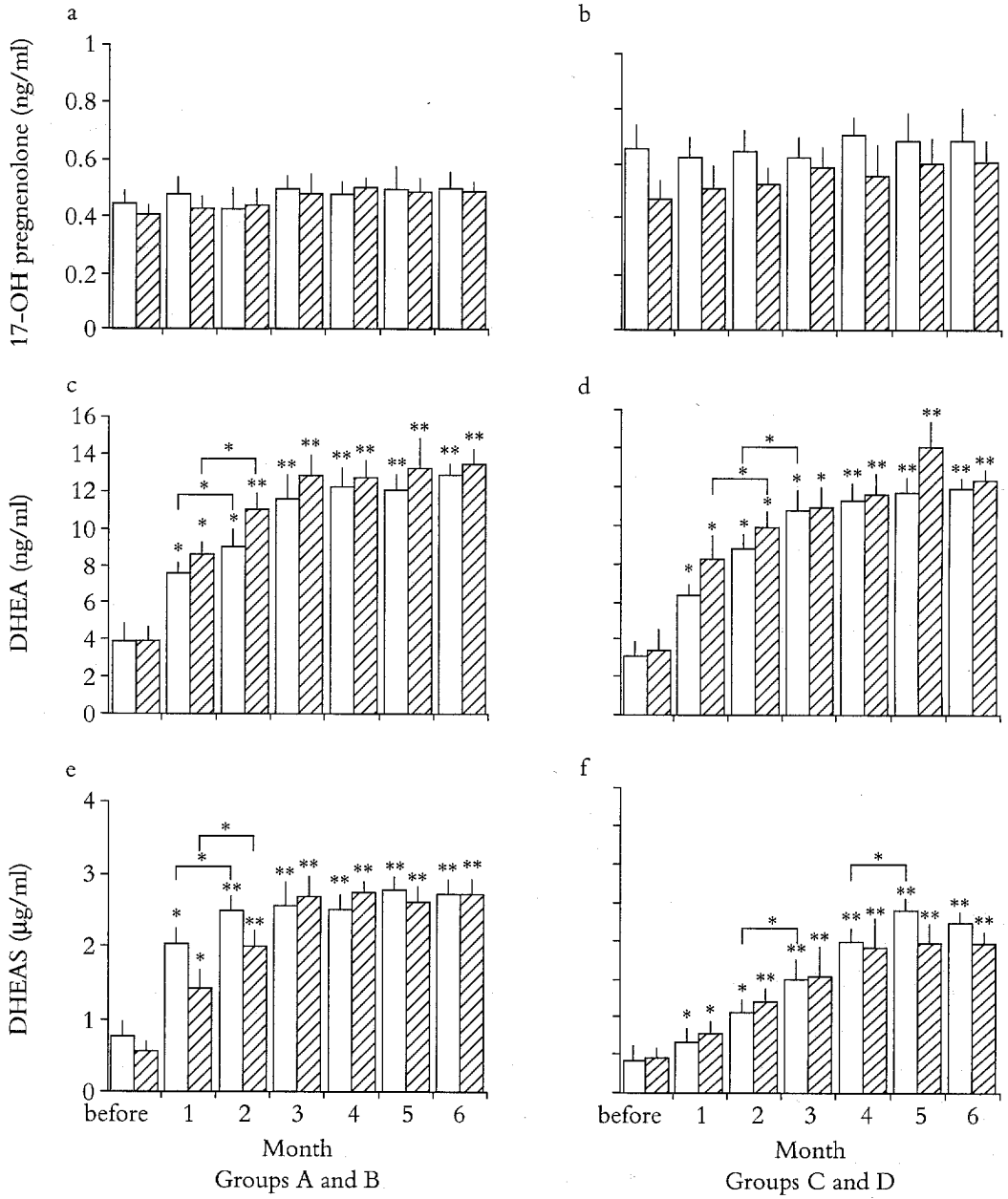

Figure 3 (a, b) Serum levels of 17-OH pregnenolone, (c, d) dehydroepiandrosterone (DHEA), and (e, f) dehydroepiandrosterone sulfate (DHEAS) in normal-weight and overweight early postmenopausal women (groups A and B) (a, c, e) and in normal-weight and overweight late postmenopausal women (groups C and D) (b, d, f), over the course of a 6-month DHEA treatment. Values are shown as mean ± SEM. □, normal weight; ▨, overweight; $*p < 0.05$; $**p < 0.01$

allopregnanolone/progesterone ratios (5α-reductase activity) showed a significant increase (Figure 1a–d). On the contrary, the cortisol/17-OH progesterone ratio (11- and/or 21-hydroxylase activities) showed a significant decrease after 6 months of treatment and the androstenedione/ DHEA ratio (3β-hydroxysteroid-oxidoreductase activity) did not vary during the treatment (Figure 1e,f).

Effects of dexamethasone suppression (Table 4) After 3 and 6 months of treatment, dexamethasone significantly suppressed all adrenal steroids; the inhibition ranged from 85 to 90% for cortisol and from 48 to 56% for allopregnanolone. In particular, the percentage of decrease of DHEA and allopregnanolone following dexamethasone was significantly higher in all groups at the third and

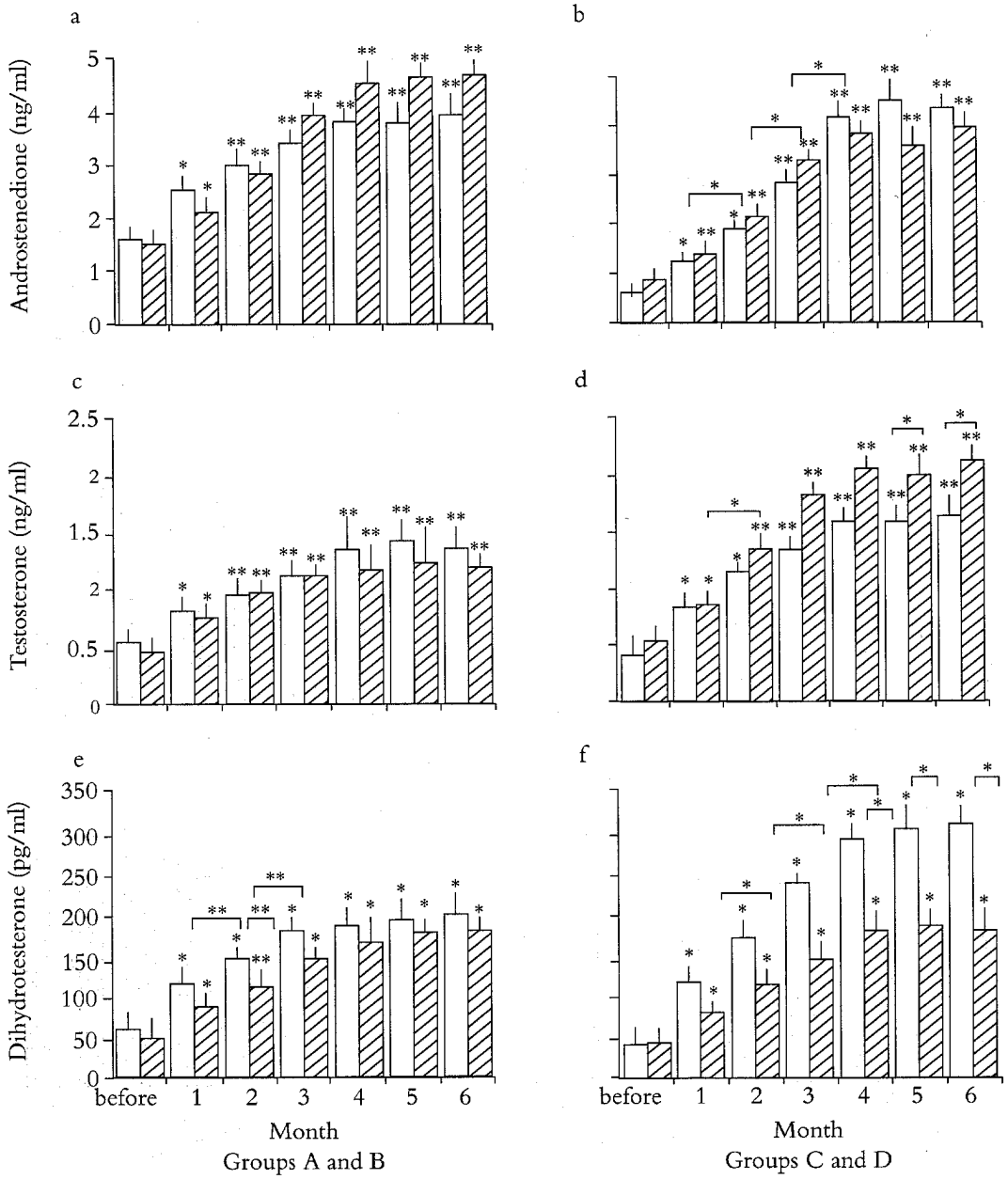

Figure 4 (a, b) Serum levels of androstenedione, (c, d) testosterone and (e, f) dihydrotestosterone in normal-weight and overweight early postmenopausal women (groups A and B) (a, c, e) and in normal-weight and overweight late postmenopausal women (groups C and D) (b, d, f) over the course of a 6-month DHEA treatment. Values are shown as mean ± SEM. ▭, normal weight; ▨, overweight; $*p < 0.05$; $**p < 0.01$

sixth month in comparison to baseline. The percentage of decrease of androstenedione was greater only in group C, while for DHEAS it was greater in groups A, C, D and, from the third month, in group B. No significant differences were observed during the treatment with regards to 17-OH pregnenolone, progesterone, 17-OH progesterone and cortisol suppressibility.

Adrenocorticotropic hormone test (Table 5) The ACTH test performed at 8.00 am, after dexamethasone suppression, induced a significant response in all parameters, showing a dynamic pattern represented, for example, for group A in Figure 2. The AUC was calculated to evaluate the differences before treatment and after 3 and 6 months of treatment in the four groups of women.

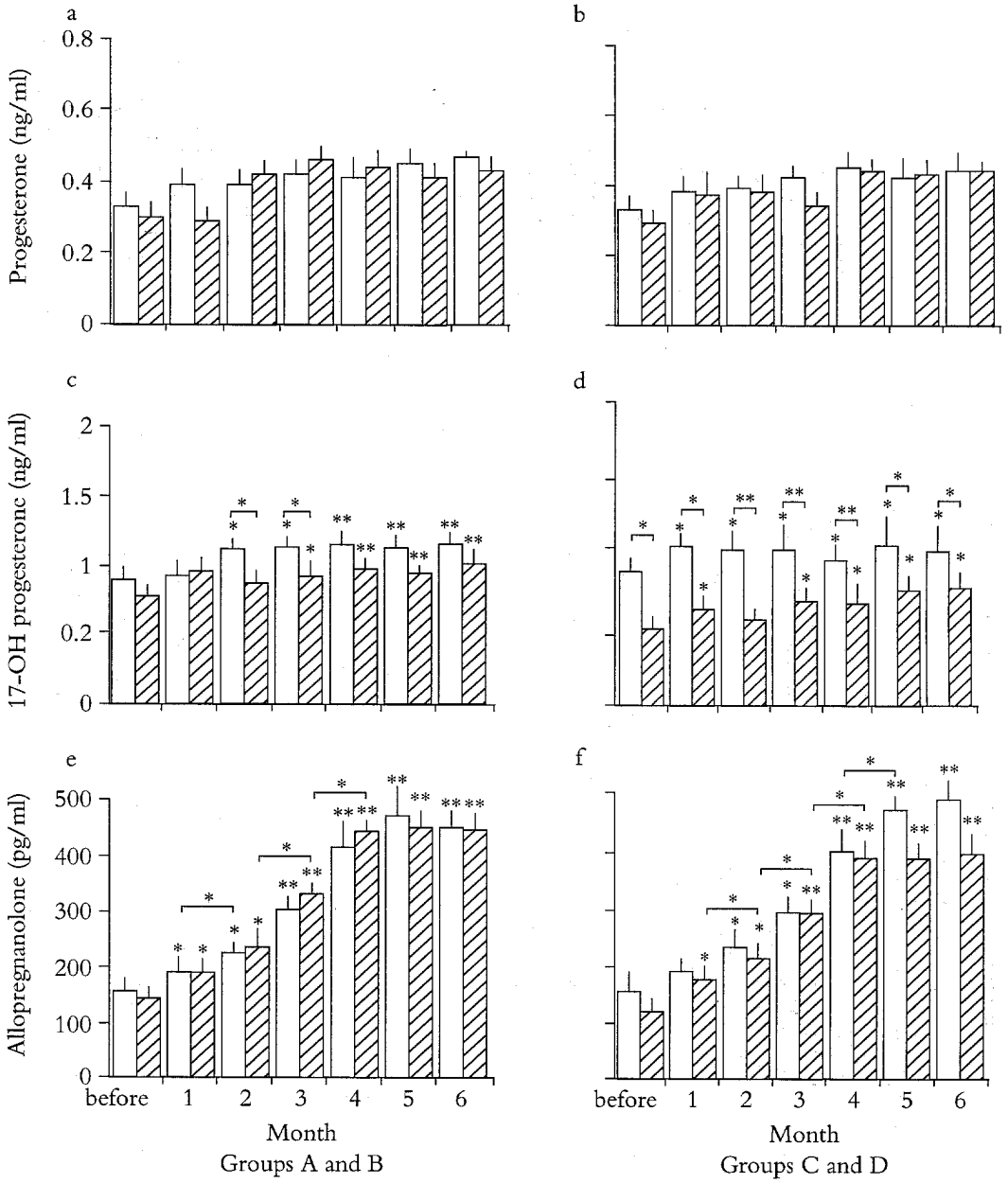

Figure 5 (a, b) Serum levels of progesterone, (c, d) 17-OH progesterone and (e, f) allopregnanolone in normal-weight and overweight early postmenopausal women (groups A and B) (a, c, e) and in normal-weight and overweight late postmenopausal women (groups C and D) (b, d, f), over the course of a 6-month DHEA treatment. Values are shown as mean ± SEM. ▭, normal weight; ▨, overweight; $*p < 0.05$; $**p < 0.01$

The 17-OH pregnenolone response to ACTH was significantly higher in group B at the sixth month; the DHEA response was significantly higher in groups A and D after 6 months, and for DHEAS in group A from the third month and in group B at the sixth month. The androstenedione response to ACTH appeared significantly higher after 3 and 6 months of treatment in all groups, while it was higher for progesterone in groups A, B and C after 3 and 6 months and in group D after 6 months, without any apparent influence of age and BMI. The 17-OH progesterone response was significantly higher after 6 months in the older normal-weight women (group C) and, after 3 months, in the overweight women (group D). The allopregnanolone response was significantly higher after 3 months in group C and after 6 months in group B, while the

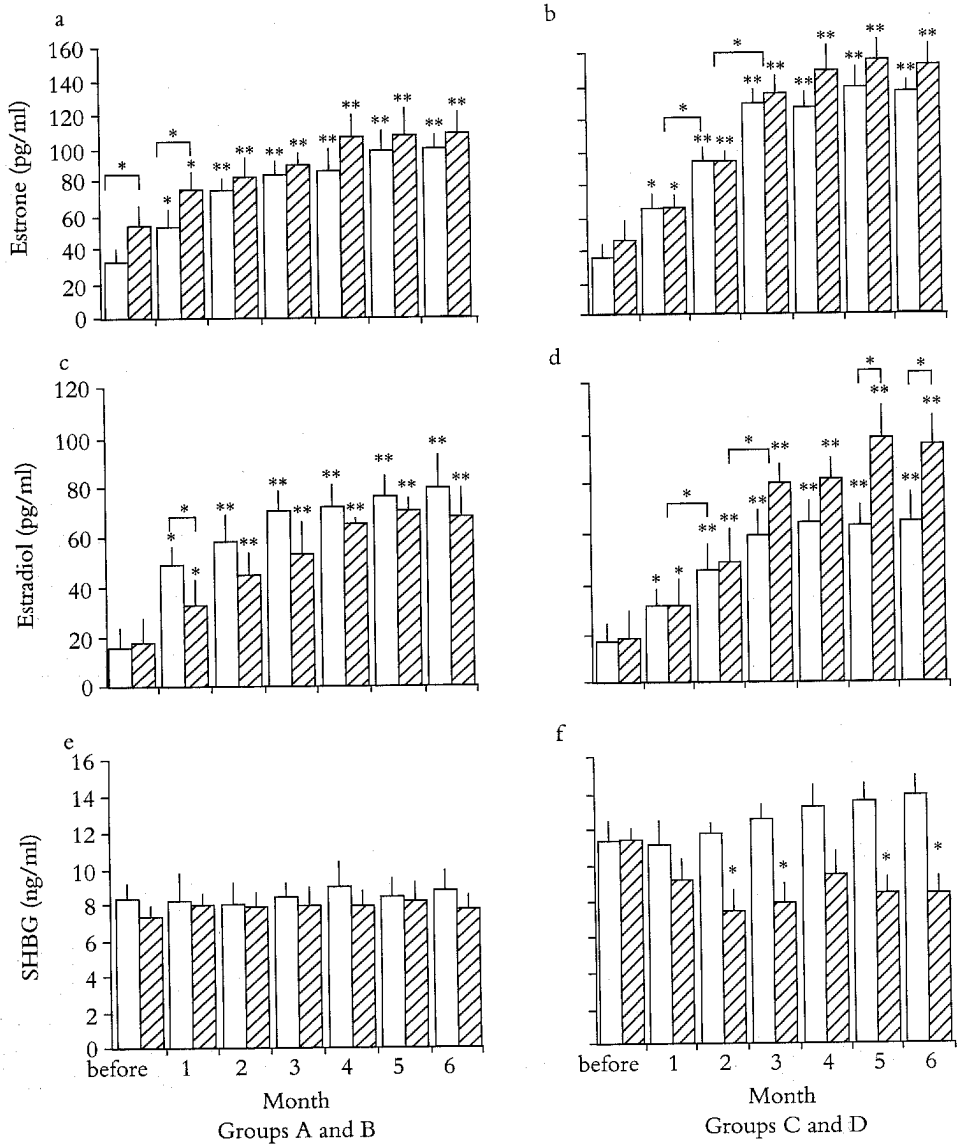

Figure 6 (a, b) Serum levels of estrone, (c, d) estradiol and (e, f) sex-hormone binding globulin (SHBG) in normal-weight and overweight early postmenopausal women (groups A and B) (a, c, e) and in normal-weight and overweight late postmenopausal women (groups C and D) (b, d, f), over the course of a 6-month DHEA treatment. Values are shown as mean ± SEM. ▭, normal weight; ▨, overweight; $*p < 0.05$; $**p < 0.01$

cortisol response to ACTH significantly decreased after 3 and 6 months in all groups, except in group D.

DISCUSSION

The present data confirm both the age-related decrease in Δ5-androgens and Δ4-androstenedione and the reduction of adrenal enzymatic activities[6,7,30–32] and show that overweight postmenopausal women have higher circulating DHEA(S) levels than normal-weight age-matched women. Mazza and colleagues[14] showed a link between BMI and DHEA(S) levels before but not after menopause, but they did not distinguish between early and late postmenopause as we have done in the present study. While circulating estradiol levels were similar in all groups, estrone values, as expected, were higher in women with higher BMI, in agreement with the relevant androgen metabolization in the adipose tissue[33]. On the contrary, in overweight women, cortisol levels

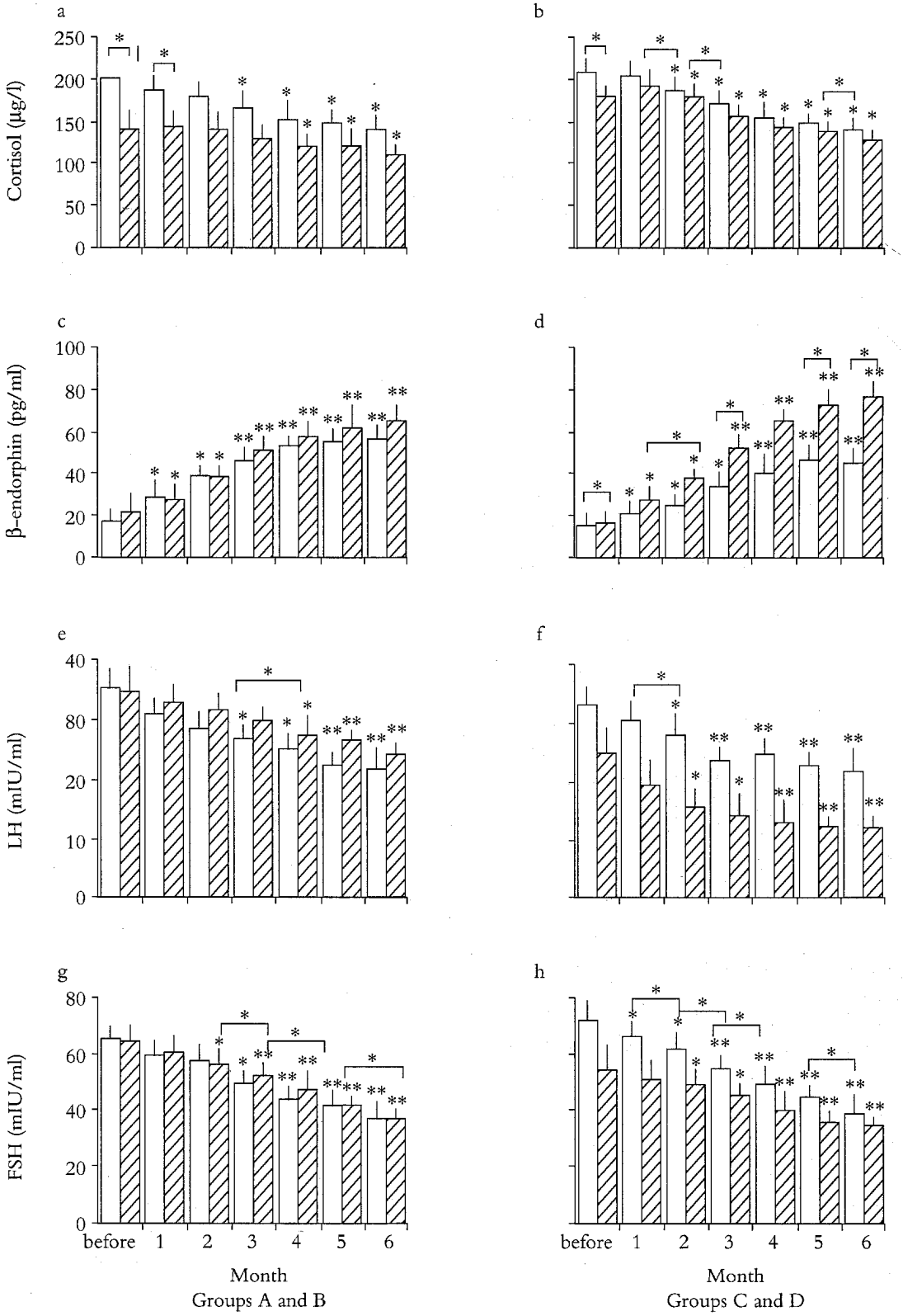

Figure 7 (a, b) Serum levels of cortisol, (c, d) β-endorphin, (e, f) luteinizing hormone (LH) and (g, h) follicle stimulating hormone (FSH) in normal-weight and overweight early postmenopausal women (groups A and B) (a, c, e, g) and in normal-weight and overweight late postmenopausal women (groups C and D) (b, d, f, h) over the course of a 6-month DHEA treatment. Values are shown as mean ± SEM. ▭, normal weight; ▨, overweight; $*p < 0.05$; $**p < 0.01$

were lower than in the age-matched normal-weight women. Our data confirm previous studies which reported a significant increase in cortisol levels related to aging (in men and women), visceral fat accumulation (in women) and carbohydrate intake (in men)[34–36], but did not show any correlation between cortisol concentrations and BMI. This may be due to the fact that only normal and overweight but not obese women (BMI > 30) were included in the present study. Lower 17-OH progesterone and allopregnanolone levels seemed to characterize the older overweight women and this data remains to be clarified. In fact, previous evidence has not revealed significant modifications in 17-OH progesterone with age and body weight, while no data are available for allopregnanolone[33]. Lately, in accordance with previous data[37], SHBG levels were found to be higher in late postmenopausal women, suggesting a lower availability of free steroids in these women.

Dexamethasone administration suppressed all the adrenal steroids and this was higher for DHEA secretion in normal-weight early postmenopausal women and for DHEAS and progesterone in normal-weight early and late postmenopausal women. Overweight women did not show any change, probably because of an increased extraglandular pool of steroids. The ACTH stimulus induced a significant response from the adrenal gland, with a greater 17-OH pregnenolone and DHEA response in younger normal-weight women; this suggests a greater sensitivity of P450 cytochrome. Levels of DHEAS responded more in normal-weight older women, suggesting a greater sulfatase activity. The lower age-related DHEA response to ACTH stimuli observed in this study is in accordance with Parker and co-workers[38]. In overweight women, progesterone levels seemed to respond more to ACTH and were suppressed to a lesser extent by dexamethasone. This finding might be related to the small number of subjects of this study.

Several reports have attempted to clarify the effects of treatment with pharmacological doses of DHEA. The first trials were performed with higher doses of the steroid (800–1600 mg) that produced supraphysiological concentrations of androgens and estrogens[15,39,40]. Studies from Yen and colleagues[19] and Young and co-workers[41], using lower doses, suggested that 50 mg of DHEA was a suitable dose for adrenal androgen replacement and Beaulieu and colleagues[25] stated that this dose produces no accumulation. Recently, investigating the effects of a 3-month oral 50 mg DHEAS supplementation in postmenopausal women, we reported that estrogens (estrone and estradiol) and androgens (DHEA(S), testosterone and androstenedione) increase to values normally present in fertile women[26].

The present data confirm the prompt increase in Δ5-androgens, with higher values starting from the fourth or fifth month. The differences among the patients observed before treatment disappeared after the first month (for DHEA) or third or fourth month (for DHEAS). The Δ4-androgens (androstenedione, testosterone and dihydrotestosterone) also increased during treatment to supraphysiological levels, with a similar trend but with some specific differences. In particular, the difference in basal androstenedione disappeared after 2 or 3 months. Testosterone levels showed significantly higher values in older overweight women, while dihydrotestosterone levels appeared higher in older women of normal weight, suggesting a greater 5α-reductase activity in this group. The estrone BMI-related difference, observed before therapy, disappeared under treatment, but the older women showed significantly higher levels of this steroid, suggesting a greater peripheral aromatase activity. These data are also supported by a previous study from Loncope[42], which showed an age-related increase in androgen aromatization (DHEA, androstenedione and testosterone) to estrone. The increase in estradiol levels was five-fold with respect to baseline evaluation, reaching values similar to those observed in women treated with 100 μg transdermal estradiol patches or 2 mg estradiol valerate[43]. This significant increase may be of relevance for the effects of estrogen on target tissues and, in particular, may antagonize the effects of postmenopausal estrogen withdrawal. Despite the elevated estrogen levels, there are apparently no significant changes in the endometrium, in the 6 months of observation, in any age group. These data are in accordance with previous studies, showing no effects of DHEA administration (in cream or orally) on the endometrium[44,45]. Labrie and co-workers[44] have suggested that the absence of the required enzymes for the transformation of DHEA into estradiol explains the absence of local stimulatory effect of

physiological doses of DHEA on the atrophic endometrium of postmenopausal women. By contrast, estrogen effects on other targets are clearly evident from the significant decrease in gonadotropins during the treatment. Before treatment, LH levels were lower in overweight older women than the other women and this trend was maintained also during the DHEA administration. This difference may be related to the estrogen levels, which were higher in these subjects with respect to the other groups.

As previously reported[26], SHBG levels did not vary over the 6 months of therapy, except for the overweight late postmenopausal women, who showed a significant reduction in SHBG levels, to values similar to those of younger subjects. The SHBG trend, under DHEA, was similar to that observed under transdermal HRT, suggesting a greater availability of free steroids for target tissues after oral DHEA[46].

Allopregnanolone levels increased significantly and progressively in all groups, reaching, at the fourth or fifth month, values similar to those observed during pregnancy[47]. This steroid is a 3-α-hydroxy-5-α-pregnane-20-one which exerts neurotropic and neuromodulatory effects in the central nervous system (CNS) and it is directly involved in modulating physical and psychological well-being, mood, and memory processes[48–50]. Allopregnanolone is also one of the steroids synthesized *de novo* from cholesterol or deriving from the metabolization of circulating precursors in the glial cells, which have been named 'neurosteroids'[48,49].

Recent data suggest that neurosteroids exert their effects by modulating the γ-aminobutyric $acid_A$ ($GABA_A$) receptor function. In particular, experimental evidence suggests that DHEA and DHEAS antagonize GABAergic activity, producing an increase in CNS neuronal excitability, while allopregnanolone acts as a $GABA_A$ receptor agonist, producing a significant increase in chloride influx into specific channels of the receptor with strong anxiolytic effects[50–53]. It is possible to speculate that the beneficial effects of DHEA(S) supplementation on sense of well-being, cognitive functions, mood and behavior may be determined by this steroid's direct action on the CNS; these effects may also be mediated by active metabolites of DHEA, or through its relevant effects on the synthesis of allopregnanolone.

Previous experimental and clinical studies have focused their attention on the role of cortisol in the physiopathological mechanisms related to the aging process. In these studies cortisol has been described as a CNS neurotoxic molecule[54,55]. The significant decrease in cortisol synthesis and release throughout the 6 months of DHEA supplementation suggests that this treatment may positively influence the organism's response to stress. This progressive decrease may be a consequence of the increase of the neurosteroid allopregnanolone. In fact, experiments in rats have shown that allopregnanolone is able to counteract the anxiogenic effects of CRF, suggesting that this neurosteroid reduces the activation of the hypothalamic–pituitary–adrenal axis, which exerts anxiolytic effects[56]. Furthermore, the decrease of cortisol prevents or attenuates the chronic degenerative CNS effects induced by elevated cortisol levels; in fact, patients affected by Alzheimer's disease and vascular dementia show elevated circulating cortisol and reduced allopregnanolone and DHEA levels[57]. On the contrary, Alzheimer's disease patients with higher endogenous DHEA or lower cortisol levels show better memory tasks than those with an opposite endogenous steroidal pattern[58]. Supposing that the impairment of the steroid pattern plays a relevant role in regulating cognitive function, DHEA supplementation in postmenopausal women may help in preventing negative CNS degenerative phenomena.

The analyses of the ratios of steroid pairs adjacent in the biosynthetic pathway demonstrated that the 17,20-desmolase activity, as revealed by the DHEA/17-OH pregnenolone ratio, is significantly increased in all groups of women under DHEA supplementation. This treatment counteracts the negative effect of aging on the enzymatic activity. Moreover, there was a significant increase in the DHEAS/DHEA ratio and this finding is in accordance with previous studies demonstrating an enhanced sulfatase and/or sulfotransferase activity. Also, the increase in 5α-reductase and the blunting of 11- and/or 21-hydroxylase activities apparently sustain changes in the intra-adrenal steroid pattern. In addition to these data, the significant rise in circulating dihydrotestosterone levels indicates an increase also in peripheral 5α-reductase activity.

The sensitivity of the adrenal gland to dexamethasone suppression increased after 3 and 6 months of treatment in terms of DHEA, DHEAS and allopregnanolone, while it remained unchanged for other steroids (cortisol, 17-OH pregnenolone, androstenedione, progesterone, 17-OH progesterone). These effects are independent of age and BMI. In younger subjects, the ACTH stimulus, after the treatment, prompted a greater response in Δ5- and Δ4-androgens, progesterone and 17-OH progesterone, while cortisol responded less in younger and in normal-weight older subjects. It seems that chorionic DHEA administration enhances some adrenal pathways and reduces the synthesis of cortisol. In younger subjects DHEA treatment may stimulate the biosynthetic pathways involved in Δ5- and Δ4-androgen synthesis and blunt cortisol production. In older subjects, DHEA treatment seems to improve the synthesis of Δ4-androgens, progesterone and 17-OH progesterone, and also blunt cortisol in normal-weight subjects. This evidence suggests that the differences obtained in the adrenal response to the ACTH stimuli are directly associated with specific effects of DHEA supplementation on the gland. The observation of a constant allopregnanolone response to ACTH in all groups, regardless of the progressive increase in its basal levels during DHEA treatment, as well as in dexamethasone suppression, suggests an important extra-adrenal production of allopregnanole during the treatment rather than a greater ACTH-induced adrenal allopregnanolone release. The presence of 5α-reductase and the 3α-OH-steroid-oxidoreductase in liver suggests that allopregnanolone may be synthesized in this organ. The increase in allopregnanolone production at the peripheral level may also be related to the enzymatic induction mediated by increased estrogen levels[48,50].

The present data confirm an increase in plasma β-endorphin levels under DHEA treatment. ACTH and β-endorphin synthesis and release are controlled by several neurotransmitters such as noradrenaline, dopamine, serotonin, acetylcholine, GABA and, in particular, CRF[59,60]. The increase in β-endorphin may be directly related to an increased cleavage of the carboxy-terminal fragment from the pro-opiomelanocortin induced by DHEA. A similar trend also occurs during puberty, a physiological situation that shows increased endogenous DHEA and β-endorphin levels associated with stable ACTH and cortisol concentration[7,61].

According to previous evidence[26], the present data showed an improvement of subjective symptoms, evaluated with the Kupperman score, in all groups of treatment. The peculiarity of these findings is the significant improvement of the score in late postmenopausal subjects with respect to the psychological symptoms, thus confirming the specific action of the Δ5-androgen, or of its different metabolites, on the neuroendocrine systems which modulate the subjective sense of well-being.

In conclusion, the present findings confirm that DHEA supplementation produces physiological and supraphysiological modifications in steroid levels and adrenal function. Furthermore, the beneficial effects of DHEA on the quality of life and in reverting aging processes may be related to changes in the release of adrenal products and/or peripheral steroids, with an increase in anxiolytic (allopregnanolone), anabolic (androstenedione, testosterone, dihydrotestosterone), estrogenic (estrone, estradiol) molecules, a beneficial decrease in cortisol and increase in circulating β-endorphin. Furthermore, previous data indicate that DHEAS supplementation in postmenopausal women restores the β-endorphin response to specific adrenergic, serotoninergic and opiatergic neuroendocrine stimuli, suggesting that the improvement of physical and psychological well-being should be in part related to a restoration of the central neurotransmitters' tonus. We believe that dehydroepiandrosterone is active as a prehormone because it produces active metabolites that induce modifications which may counteract the negative phenomena related to menopause and aging. By consequence, DHEA should be considered as medication, not simply as a dietary supplement, and should, therefore, not be freely accessible to the public as it is now. More extensive studies are required to confirm the lack of effects on the endometrium and the breast, and to evaluate any alterations in metabolic parameters and also the efficacy of lower doses of the drug.

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