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Long-term low-dose dehydroepiandrosterone replacement therapy in aging males with partial androgen deficiency

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ABSTRACT

Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) age-related withdrawal is very likely to be involved in the aging process and the onset of age-related diseases, giving rise to the question of whether preventing or compensating the decline of these steroids may have endocrine and clinical benefits. The aim of the present trial was to evaluate the endocrine, neuroendocrine and clinical consequences of a long-term (1 year), low-dose (25 mg/day) replacement therapy in a group of aging men who presented the clinical characteristics of partial androgen deficiency (PADAM). Circulating DHEA, DHEAS, androstenedione, total testosterone and free testosterone, dihydrotestosterone (DHT), progesterone, 17-hydroxyprogesterone, allopregnanolone, estrone, estradiol, sex hormone binding globulin (SHBG), cortisol, follicle stimulating hormone (FSH), luteinizing hormone (LH), growth hormone (GH) and insulin-like growth factor 1 (IGF-1) levels were evaluated monthly to assess the endocrine effects of the therapy, while β-endorphin values were used as a marker of the neuroendocrine effects. A Kupperman questionnaire was performed to evaluate the subjective symptoms before and after treatment.

The results showed a great modification of the endocrine profile; with the exception of cortisol levels, which remained unchanged, DHEA, DHEAS, androstenedione, total and free testosterone, DHT, progesterone, 17-hydroxyprogesterone, estrone, estradiol, GH, IGF-1 and β-endorphin levels increased significantly with respect to baseline values, while FSH, LH and SHBG levels showed a significant decrease. The Kupperman score indicated a progressive improvement in mood, fatigue and joint pain.

In conclusion, the present study demonstrates that 25 mg/day of DHEA is able to cause significant changes in the hormonal profile and clinical symptoms and can counteract the age-related decline of endocrine and neuroendocrine functions. Restoring DHEA levels to young adult values seems to benefit the age-related decline in physiological functions but, however promising, placebo-controlled trials are required to confirm these preliminary results.

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INTRODUCTION

Dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS), the most abundant circulating steroids, are secreted by the adrenal cortex in response to adrenocorticotropin and by other minor sources such as the gonads and the placenta. Despite the fact that the specific biosynthetic enzyme complex 17α-hydroxylase-17-20-desmolase has not been localized in the brain[1], DHEA(S) is produced in the central nervous system, where it reaches concentrations higher than those present in plasma[2,3] and affects the GABAergic and glutaminergic neurotransmission directly by binding to neural membrane receptors[4–6]. Moreover, it has been recently demonstrated that DHEA(S) can bind to a specific plasma membrane receptor coupled to $G\alpha i_{2,3}$[7], leading to the MAPK signaling cascade and to rapid nitric oxide synthesis in endothelial cells[8].

The exact mechanisms by which these steroids act are unknown and it is strongly hypothesized that DHEA(S) can have indirect effects in many tissues, since its metabolic transformation gives rise to active steroids such as androgens and estrogens[9].

Sex, smoking, weight and dietary intake have been associated with changes in serum DHEA(S) values[10,11]; however, the factor that most strongly seems to influence the concentration of these hormones is age. DHEA(S) production begins during fetal life from the 'fetalis zona' of the adrenals, whose unexplained regression after birth is associated with a consistent fall in the levels of these steroids. The development of the adrenal gland's reticularis zona determines a significant increase in circulating levels of DHEA(S) at the beginning of the second decade of life, leading to those physical and endocrine changes called 'adrenarche' (followed by a progressive increase in DHEA and DHEAS concentrations).

From the third decade of life, adrenal production decreases linearly, with a parallel reduction in DHEA(S) levels. At the age of 80 years, DHEA(S) levels are equal to 10–20% of the concentrations present in young adults[12,13]. Moreover, there are significant individual differences in the rate of DHEA(S) decline, suggesting that this process may be controlled genetically[14].

The lowering of DHEA(S) levels has been associated with breast cancer in women[15,16], increased cardiovascular mortality in men[17,18], a decline in immunocompetence[19], and changes in the amount or distribution of body fat[20]. Moreover, there is evidence for improvement in psychological well-being following DHEA therapy in aging men and women[21] and in relatively young patients affected by Addison disease[22].

The controversies about a potential pathogenetic role in Alzheimer's disease have not yet been solved[23,24]. Experimental trials report a neuroprotective and neurotropic effect of DHEA on neurons and glial cells[25,26], but no study has clearly correlated DHEA(S) to the prevalence of this disease. It seems that the imbalance of the DHEA(S)/cortisol ratio may play some role in both the degeneration of neurons and in the decline in immune function. DHEA(S), in fact, has a potent antiglucocorticoid action. However, due to its age-related decrease, as time passes, it will always counteract the immunosuppressive and neurotoxic effects of cortisol to a lesser extent[27,28].

On these bases, the question arises whether preventing or correcting the fall in DHEA(S) levels by exogenous supplementation can avoid or reverse some of the age-related changes.

The first intriguing aspect of the numerous trials on DHEA replacement concerns the complex biochemical effects of this supplementation. DHEA treatment seems to modify many endocrine and metabolic parameters.

Second, results yielded from various studies are very discordant due to differences in doses, routes of administration and duration of the treatment, and characteristics of the participants. Gender, age, and genetic background are factors that can influence both endogenous DHEA(S) levels and the metabolism of exogenous DHEA. Therefore, one of the main problems linked to DHEA replacement therapy is individualizing the adequate dose and the proper duration. None of the clinical studies, including those using supraphar-macological doses (1600 mg/day) of DHEA[29] have ever shown direct acute or subacute side-effects. However, given the effects of DHEA on the endocrine system and the fact that the intracrine metabolism cannot be quantified by measuring steroid serum levels[9], side-effects associated to chronic therapy cannot be excluded.

The aim of the present study was to analyze the clinical and endocrine effects of a long-term, low-

dose (25 mg/day) DHEA replacement therapy in aging men who presented the clinical and hormonal characteristics of partial androgen deficiency of aging male (PADAM)[30].

The endocrine evaluation consisted in verifying the effects of DHEA therapy on gonadal, somatotropic and adrenal axes. Furthermore, the neuroendocrine effects were evaluated by assessing circulating β-endorphin levels, a marker of neuroendocrine function[31].

MATERIALS AND METHODS

Subjects

Ten elderly men (age range 58–69 years), presenting the PADAM symptoms complex[30] (reduction of libido, decline in muscular mass and strength, apathy, arthralgia and ostalgia), with decreased serum levels of total testosterone (<2.5 ng/ml) or free testosterone (<24 pg/ml), were recruited for this trial.

All the subjects had a normal weight (body mass index 20.4–22.7 kg/m^2), were non-smokers, and healthy on the basis of medical history, physical examination, blood chemistry profile and blood count. Psychiatric disorders, endocrine diseases, renal and liver disorders were excluded; they were taking no medications in the 6 months prior to the beginning of the treatment and had a stable diet.

This study was prospective and lasted 12 months; all subjects were given 25 mg of DHEA every morning at 8.00. All subjects completed the protocol. The local Ethics Committee approved the study and a written informed consent was obtained from each of the participants before study initiation.

Protocol

The subjects were evaluated monthly through blood sampling. Well-being was assessed by completing a Kupperman questionnaire before and at study termination. Blood samples were collected between 8.00 and 9.00 after overnight fasting, 24 h after the last DHEA daily dose, in order to assess the circulating levels of DHEA, DHEAS, 17-hydroxyprogesterone, progesterone, cortisol, growth hormone (GH), insulin-like growth factor 1 (IGF-1), luteinizing hormone (LH), follicle stimulating hormone (FSH), estrone, estradiol, androstenedione, total testosterone, free testosterone, dihydrotestosterone (DHT), sex hormone binding globulin (SHBG), allopregnanolone and β-endorphin. The specimens were centrifuged and serum or plasma was stored at -20°C until assay.

The Kupperman questionnaire was used to evaluate subjective complaints for neurovegetative and psychological symptoms. Subjects were asked to quantify each symptom through a rating scale whose range was from 0 ('none') to 3 ('marked').

Before and at study termination, a transrectal ultrasound was performed to evaluate the effect of the DHEA administration on prostatic tissue.

Assay

Serum concentrations of DHEA, DHEAS, androstenedione, total and free testosterone, DHT, cortisol, 17-hydroxyprogesterone, progesterone, estrone, estradiol, LH, FSH, GH, and SHBG were measured by specific commercially available radioimmunoassay kits (Radim®; Pomezia, Rome, Italy). The sensitivities of the assays were 0.2 ng/ml for DHEA, 0.02 μg/ml for DHEAS, 0.03 ng/ml for androstenedione, 0.017 ng/ml for total testosterone, 0.18 pg/ml for free testosterone, 4 pg/ml for DHT, 0.9 μg/l for cortisol, 0.1 ng/ml for 17-hydroxyprogesterone, 0.12 ng/ml for progesterone, 10 pg/ml for estradiol, 12 pg/ml for estrone, 0.20 mIU/ml for LH, 0.18 mIU/ml for FSH, 0.04 ng/ml for GH, 3.0 ng/ml for SHBG. The intra- and interassay coefficients of variation were, respectively, 3.8% and 6.9% for DHEA, 4.0% and 8.5% for DHEAS, 4.3% and 6.0% for androstenedione, 5.1% and 7.8% for total testosterone, 3.7% and 7.3% for free testosterone, 4.5% and 8.4% for DHT, 3.6% and 6.2% for cortisol, 4.5% and 6.1% for 17-hydroxyprogesterone, 4.8% and 9.2% for progesterone, 2.1% and 3.5% for estradiol, 4.4% and 6% for estrone, 2.8% and 3.3% for LH, 1.97% and 4.11% for FSH, 3.4% and 5.0% for GH, and 4.6% and 5.9% for SHBG.

IGF-1 concentrations were determined with the use of a radioimmunoassay kit (Medgenix, Fleurus, Belgium) after acid-ethanol extraction, as described by Daughday and colleagues[32]. The sensitivity of the assay was 0.8 ng/ml and the

intra-assay and interassay coefficients of variation were 3.0% and 3.7%, respectively.

Allopregnanolone measurement was performed after ether extraction and chromatographic partition on Sep-Pak C18 cartridges (Waters Co., Milford, USA) using a specific previously described radioimmunoassay method[33]. The sensitivity of the assay was 10 pg/tube and the intra- and interassay coefficients of variation were 7.2% and 9.1%, respectively.

β-endorphin evaluation was performed using a specific previously described radioimmunoassay[34] after its extraction and chromatographic partition by using Sep-Pak C18 cartridges; the sensitivity was 2.5 pg/ml and the intra- and interassay coefficients of variation were 6% and 9%, respectively.

Statistics

The data obtained were expressed as mean $\pm$ standard deviation and were compared by using a paired Student's *t* test.

RESULTS

Clinical findings

During the treatment no abnormal events or side-effects were recorded. Ultrasound evaluation of the prostatic tissue after 12 months of DHEA therapy did not show any significant modification with respect to pretreatment findings.

The clinical symptoms showed a significant improvement in mood, fatigue and joint pain, as evidenced by Kupperman score evaluation before and after treatment (Figure 1).

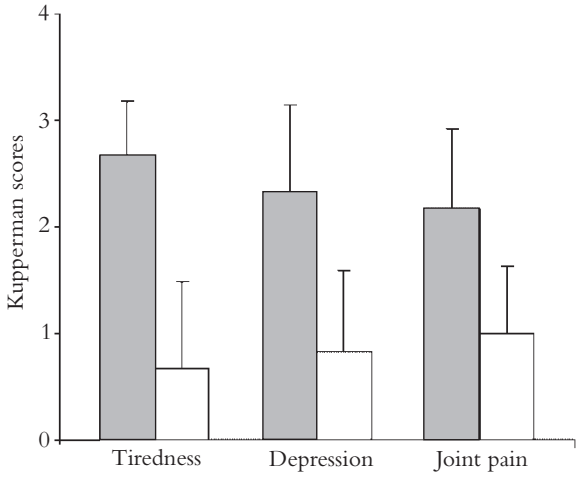

Figure 1 Comparison of the Kupperman scores before (gray column) and after (white column) DHEA treatment. $\star p < 0.05$; $\star\star p < 0.01$; $\star\star\star p < 0.001$

Hormonal evaluation

In general, the comparison with baseline hormone levels showed that DHEA supplementation induces a deep change in all hormonal parameters considered, with the exception of cortisol.

DHEA, DHEAS, androstenedione, total testosterone, free testosterone and DHT (Figure 2)

Pretreatment DHEA (2.29 ± 0.59 ng/ml) and DHEAS (0.53 ± 0.20 μg/ml) concentrations were in the low age-related range. DHEA serum levels increased significantly from the first month ($p < 0.05$) of therapy, reaching two-fold levels (5.52 ± 1.16 ng/ml, $p < 0.001$) at the 8th month. DHEA levels did not show further significant changes thereafter. DHEAS levels increased significantly at the 1st month ($p < 0.05$) and reached values 2.5 times higher (1.68 ± 0.59 μg/ml, $p < 0.01$) at the 7th month of therapy, remaining stable thereafter.

Androstenedione mean levels (0.81 ± 0.18 ng/ml) showed a progressive increase, which became significant at the 4th month of treatment ($p < 0.05$) and reached the steady state at the 8th month (1.51 ± 0.35 ng/ml). Total testosterone (2.20 ± 0.35 ng/ml), free testosterone (18.08 ± 5.52 pg/ml) and dihydrotestosterone (29.66 ± 7.84 pg/ml) levels increased significantly ($p < 0.05$) at the 5th month of therapy and reached the steady state at the 7th month (3.61 ± 0.34 ng/ml; 41.55 ± 5.95 pg/ml; 56.33 ± 8.45 pg/ml, $p < 0.01$).

Progesterone, 17-hydroxyprogesterone, allopregnanolone, estrone, estradiol, SHBG (Figure 3)

Progesterone levels (0.23 ± 0.11 ng/ml) and 17-hydroxyprogesterone levels (0.58 ± 0.20 ng/ml) showed a progressive increase, which became significant at the 4th month of therapy ($p < 0.05$), and reached highest values at the 8th month, remaining stable thereafter (0.59 ± 0.15 ng/ml and 0.89 ± 0.21 ng/ml, respectively, $p < 0.01$).

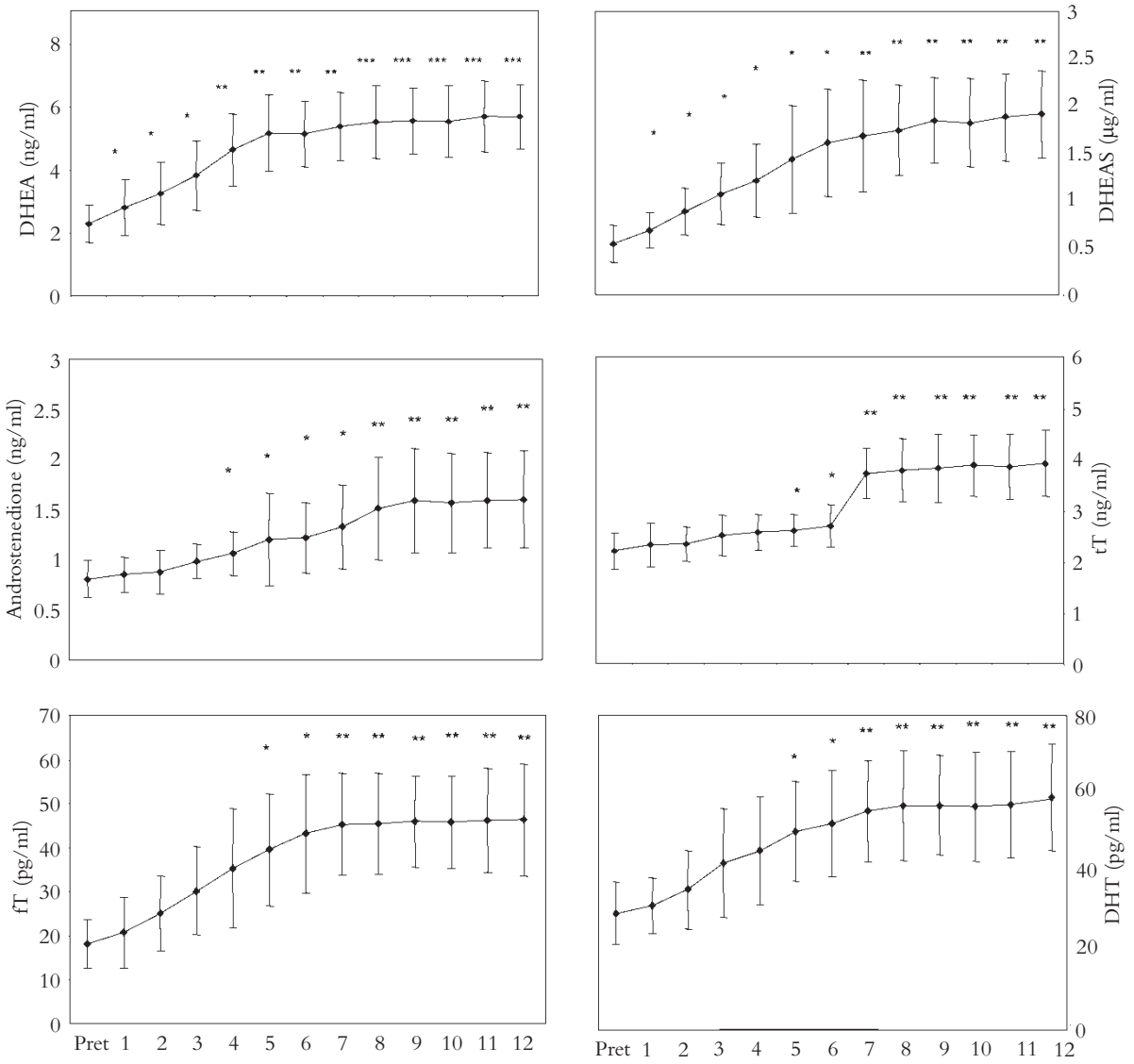

Figure 2 Serum levels of DHEA, DHEAS, androstenedione, total (tT) and free testosterone (fT), and DHT during the 12 months of DHEA therapy. ⋆$p < 0.05$; ⋆⋆$p < 0.01$; ⋆⋆⋆$p < 0.001$

Allopregnanolone levels (163.8 ± 38.6 pg/ml) rose significantly at the 7th month ($p < 0.05$) and reached the steady state at the 9th month (269.8 ± 49.3 pg/ml, $p < 0.001$). SHBG values (17.0 ± 2.4 ng/ml) showed a significant decrease from the 7th month of therapy (14.94 ± 1.34 ng/ml, $p < 0.05$). Estrone levels (34.17 ± 12.19 pg/ml) increased progressively from the 4th month ($p < 0.05$) and reached the steady state at the 8th month of therapy (70.5 ± 12.9 pg/ml). Estradiol levels (18.67 ± 3.08 pg/ml) rose significantly at the 3rd month of therapy ($p < 0.05$) and reached the steady state at the 9th month (43.5 ± 8.09 pg/ml, $p < 0.001$).

FSH, LH, GH, IGF-1, β-endorphin and cortisol (Figure 4)

FSH levels (4.85 ± 0.95 mIU/ml) showed a significant and progressive decrease from the 4th month ($p < 0.05$) to the 12th month of DHEA therapy (3.41 ± 0.68 mIU/ml, $p < 0.01$). LH levels (3.69 ± 1.65 mIU/ml) decreased significantly at the 7th month of therapy ($p < 0.05$) and remained constant until the end of treatment. GH (0.60 ± 0.21 ng/ml) rose significantly at the 4th month of therapy ($p < 0.05$) and reached values 1.5 times higher at the 10th month (0.93 ± 0.24 ng/ml, $p < 0.001$). IGF-1 levels

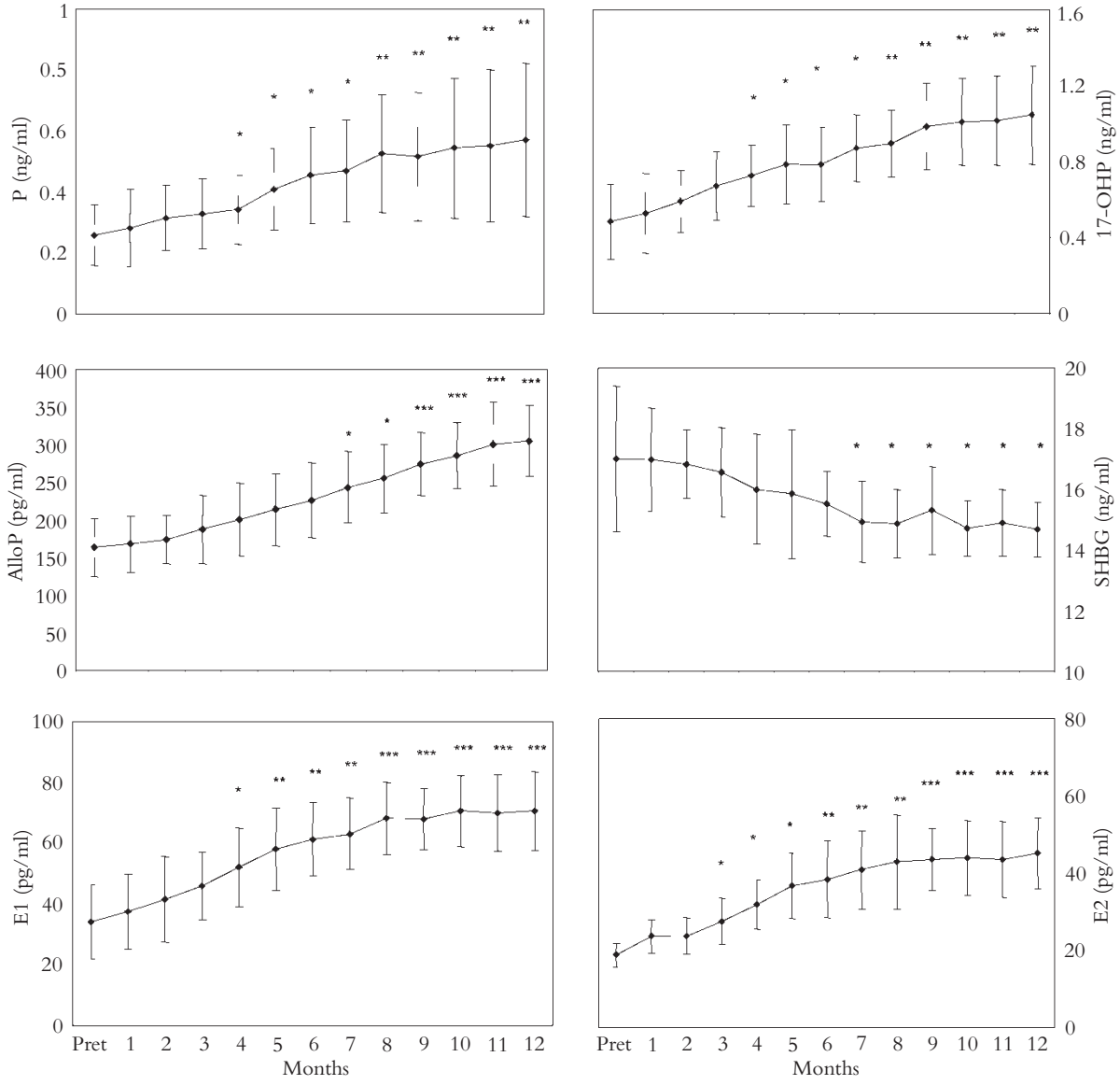

Figure 3 Serum levels of progesterone (P), 17-hydroxyprogesterone (17-OHP), allopregnanolone (AlloP), estrone (E1), estradiol (E2) and SHBG during the 12 months of DHEA therapy. ⋆$p < 0.05$; ⋆⋆$p < 0.01$; ⋆⋆⋆$p < 0.001$

(70.95 ± 16.57 ng/ml) showed a significant increase at the 5th month of treatment ($p < 0.05$), reaching highest values (110.57 ± 15.36 ng/ml, $p < 0.001$) at the 9th month, and remained stable thereafter.

β-endorphin levels (10.3 ± 4.2 pg/ml) showed a significant increase at the 6th month ($p < 0.05$) and reached the steady state at the 9th month (18.1 ± 4.9 pg/ml, $p < 0.01$). Cortisol basal levels (152.2 ± 25.7 μg/l) did not show any significant variation throughout the treatment.

DISCUSSION

The present data reveal that almost all the measured hormones are affected by DHEA supplementation. This finding was expected, considering that the synthesis of DHEA is one of the first steps in the complex steroidogenic chain. However, the greater availability of precursors is not a sufficient enough reason to justify the rise in concentration of certain hormones. In fact, not only were the end-products increased, but other biosynthetic steps were enhanced as

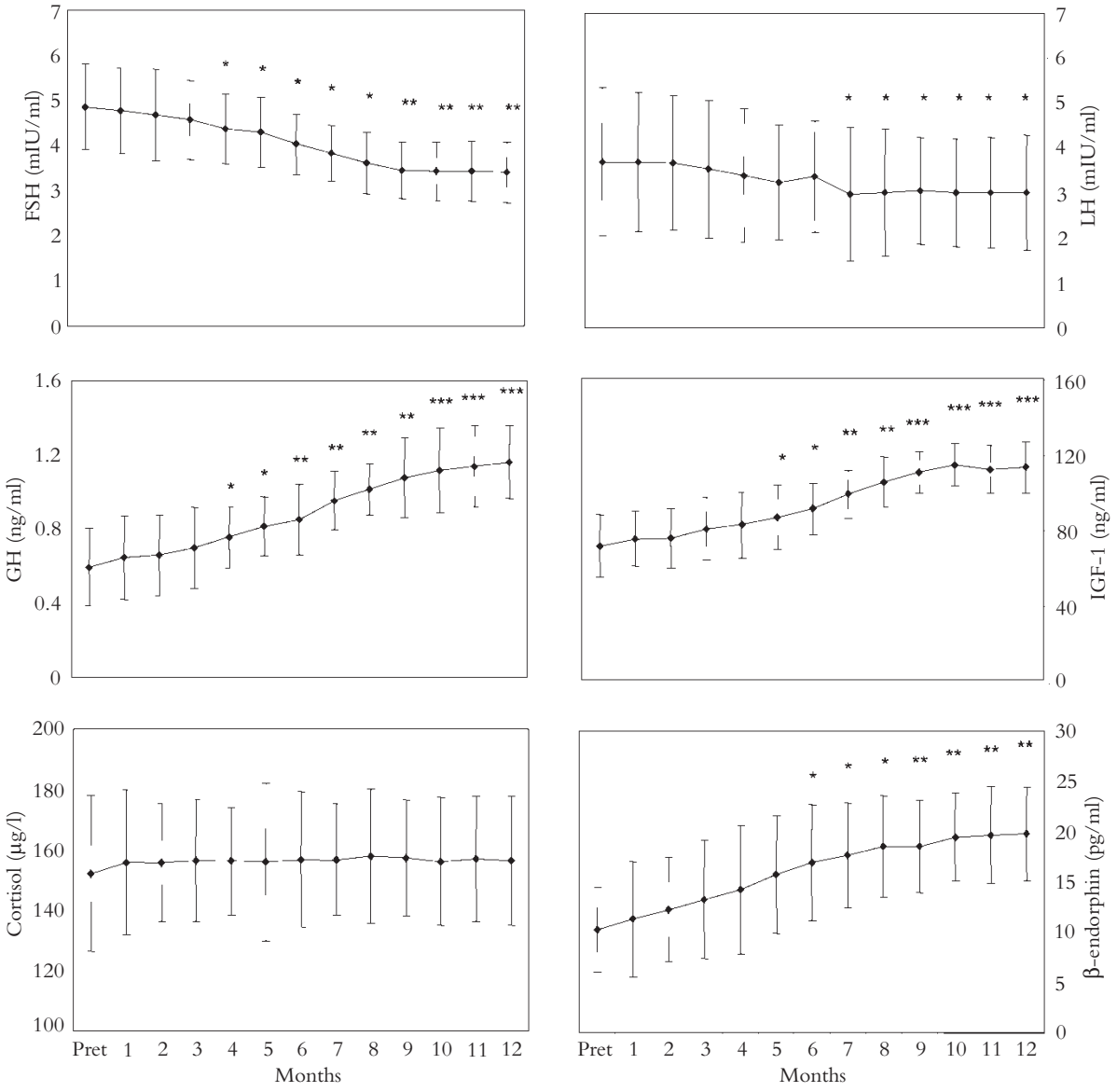

Figure 4 Serum levels of LH, FSH, GH, IGF-1, cortisol and β-endorphin during the 12 months of DHEA therapy. $\star p < 0.05$; $\star\star p < 0.01$; $\star\star\star p < 0.001$

well, as shown by the significant increase in progesterone, 17-hydroxyprogesterone and allopregnanolone levels. Moreover, other products of non-steroidal origin underwent significant variations. The explanation for the multiple effects on hormonal parameters could be that DHEA administration produces a new endocrine milieu, thus inducing other specific reactions and mutual interactions involving either different intra-adrenal enzymatic activity as well as non-steroidal hormones. In accordance with previous studies[35], the present findings confirm that the dose of 25 mg/day is able to restore DHEA and DHEAS levels into the range of young adult values. However, except for the rise in androstenedione levels, the effects obtained on the androgenic pool of steroids are discordant with data from literature[21,36,37]. Our results in aging men with partial androgen deficiency show an increase in total testosterone, free testosterone and dihydrotestosterone values after the 5th month of DHEA therapy, while several studies report that an increment in Δ4-steroids induced by DHEA supplementation occurs only in women[38]. The

reason for such a discrepancy could be due either to the difference in duration of therapy, since the previous trials performed shorter supplementations, although with higher doses, or to the fact that this study was performed on a group of men presenting clinical and hormonal signs of PADAM.

In fact, these subjects entered the study with testosterone levels lower than 2.5 ng/ml and DHEA and DHEAS levels equal to 2.29 ng/ml and 0.53 μg/ml, respectively. During DHEA supplementation, testosterone levels rose to 3.6 ng/ml; DHEA and DHEAS levels increased to 5.52 ng/ml and 1.68 μg/ml, respectively. Baulieu and colleagues[38] performed a study with a higher dose (50 mg/day) administered to men with normal baseline total testosterone levels (three-fold higher than our group) and no significant modification in its serum levels were observed at 6 and 12 months. The extremely different baseline conditions may explain the differences in study results: one can suppose that a significant hypogonadism may influence the effect of DHEA supplementation on gonadal and adrenal steroidogenesis, while a further rise in circulating testosterone in eugonadal men may determine a decrease in gonadal testosterone production due to a negative feedback mechanism on behalf of gonadal steroid products. Moreover, one may hypothesize that long-term DHEA therapy is able to restore the synthesis of androgens in Leydig cells in PADAM patients, as also demonstrated by the observed reduction in circulating gonadotropins in our patients after long-term DHEA therapy.

In men, basal testosterone secretion has been shown to be independent from that of DHEAS[39], since the former is mostly testicular and under gonadotropin control, while the latter is mainly adrenal and ACTH-regulated. It seems that DHEA may be converted into testosterone, as suggested by experimental studies[40] and by the parallel age-related decline in their circulating levels. Moreover, as reported by Valenti and colleagues[41], the deep change that occurs in the endocrine milieu during the aging process may underlie a shift in tissue metabolism and, thus, the restoration of DHEA to young adult levels may modify intracrine activity to compensate for the lack in testosterone. The rise in androgen levels can only partially justify the results concerning gonadotropin levels: the small but significant decrease in FSH levels is more probably due to a direct and specific influence on the testis' endocrine function. In the male, LH pituitary secretion is influenced by the testosterone negative feedback, while FSH levels are mainly under the control of testicular inhibin. Surprisingly, FSH levels significantly decreased before the rise in testosterone values, suggesting that the testicular intracrine balance may be affected by chronic DHEA administration.

Both estradiol and estrone levels showed a significant increase during the treatment. The rise in estrogens, although not confirmed in all studies, seems to be independent from that of androgens and is assumed to contribute to the beneficial effects of DHEA therapy in elderly men[42]. The estrogenic milieu probably underlies the positive modulation of the therapy on the somatotropic axis. DHEA administration in postmenopausal women acts on the GHRH–GH–IGF-1 axis in a manner comparable to estrogen–progestin replacement therapy[43]. In men, exogenous DHEA and/or the steroid metabolites derived from its transformation may influence the spontaneous GH pulsatile release, justifying the significant increase in GH and IGF-1 levels. The important role of estrogens and androgens in the modulation of pituitary GH secretion may explain the discordance with Morales' data[21,37], since neither estrogens nor androgens underwent significant variations in his trial. Moreover, that DHEA may act directly on hepatic IGF-1 production, independently from GH levels, cannot be excluded.

β-endorphin belongs to the endogenous opioid system and is involved in the modulation of mood, analgesia, thermoregulation and reproductive function. While its pituitary secretion is strictly linked to that of ACTH, the increase in β-endorphin values determined by DHEA administration is not associated with changes in cortisol secretion. DHEA may interfere with the cleavage of the carboxy-terminal fragment of the pro-opiomelanocortin, as probably occurs at puberty[44], when DHEA and β-endorphin levels increase while ACTH and cortisol remain constant. Moreover, DHEA supplementation in postmenopausal women has been shown to correct the age-related decline of the adrenergic, serotoninergic and opioidergic control of pituitary

β-endorphin secretion[45]. DHEA supplementation may influence β-endorphin secretion in response to specific neuroendocrine stimuli in men as well, but this hypothesis needs further investigation.

Peripheral sources of allopregnanolone, the tetrahydroderivative of progesterone, are the adrenals in men and both adrenals and ovaries in women. The peripheral production of allopregnanolone is mainly under the control of corticotropin and gonadotropin. Allopregnanolone is also synthesized by the glial cells in the brain, where it acts as $GABA_A$ receptor agonist. The brain synthesis of this molecule seems to be positively influenced by estradiol; it has been reported that estradiol induces the activity of 3α-hydroxysteroid oxydoreductase in rat brain[46]. Our recent data indicate that, in postmenopausal women, hormone replacement therapy induces a significant rise in circulating allopregnanolone levels, suggesting that estrogens activate the enzymatic pattern responsible for adrenal synthesis of allopregnanolone[47]. These observations lead us to speculate that the significant increase in allopregnanolone levels observed in PADAM men after DHEA supplementation can be due to the enzymatic induction of the allopregnanolone biosynthetic pathway, either directly by DHEA or through the DHEA-induced rise in estrogen and progesterone levels. Moreover, the fact that the increase in circulating levels of progesterone (4th month) precedes that of allopregnanolone (7th month) during the treatment further supports the hypothesis that progesterone can act as a precursor and cause allopregnanolone increase.

Thanks to the results obtained with the Kupperman questionnaire, the present trial has demonstrated that DHEA therapy may improve psychological symptoms in PADAM, although these data need to be confirmed by a placebo-controlled study. In the study by Artl and colleagues[48], however, no significant modifications in well-being or sexuality scores were observed after 4 months of 50 mg/day DHEA supplementation in a group of asymptomatic men with normal testosterone baseline levels. A recent study[49] has also demonstrated no effect of DHEA supplementation (50 mg/day for 12 months) on muscle strength in men and women aged 60–80 years. These data, combined with ours, lead us to think that the clinical effects of DHEA supplementation may be more evident in men who suffer from partial androgen deficiency (PADAM).

In conclusion, DHEA supplementation certainly has a potential in the prevention and treatment of age-related diseases and physiological decline of endocrine and neuroendocrine functions. The link between DHEA and the aging process, suggested by the age-related withdrawal of this steroid, is supported by the evidence that, in PADAM, the return to young adult DHEA levels is even able to counteract the age-related decline of other endocrine systems such as the somatotropic and gonadal axis and the neuroendocrine system.

However, more trials need to be performed to understand the possible effects of DHEA supplementation in the aging male and to distinguish the effects of the treatment from experimental biases such as subject selection (PADAM) and individual factors.

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