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Oral dehydroepiandrosterone restores β -endorphin response to OGTT in early and late postmenopause

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ABSTRACT

β -endorphin is a neuropeptide involved in several brain functions: its plasma levels are higher in obese women and its release increases after oral glucose tolerance test (OGTT) in normal or obese women. The study included 46 healthy women and evaluated the effect of oral dehydroepiandrosterone [DHEA] (50 mg/day) in early postmenopausal women (50–55 years) both of normal weight (group A, $n = 12$, BMI = 22.1 ± 0.5) and overweight (group B, $n = 12$, BMI = 28.2 ± 0.5), and late postmenopausal women (60–65 years) both normal weight (group C, $n = 11$, BMI = 22.5 ± 0.6) and overweight (group D, $n = 11$, BMI = 27.9 ± 0.4) undergone OGTT, in order to investigate if DHEA could restore/modify the control of insulin and glucose secretion and β -endorphin release in response to glucose load. The area under the curve (AUC) of OGTT evaluated plasma levels of different molecules. DHEA, DHEAS, and β -endorphin plasma levels were lower in baseline conditions in older women than younger women. Considering the AUC of β -endorphin response to OGTT, all groups showed a progressive significant increase after 3 and also after 6 months of treatment in comparison to baseline and 3 months of treatment.

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Introduction

Menopause represents a peculiar moment of women's life. After 70 years of age, dehydroepiandrosterone [DHEA(S)] levels are 20% or less of the maximum plasma concentrations [1]. It has been hypothesized that during the aging process, the reduction of 17,20 desmolase activity, the enzyme that rules the biosynthesis of the $\Delta 5$ -adrenal pathway, may induce modifications in DHEA(S) synthesis [2–6]. Growing evidence in literature support the hypothesis that lower levels of DHEA occurring during aging have been associated with impaired glucose tolerance, insulin resistance, and diabetes [7,8]. Moreover, recent data demonstrated that DHEA administration greatly improves most of menopausal symptoms and endocrine impairments [4,5,9,10] acting on each tissue and organ through an intracrinological effect since this molecule acts as a sort of pre-hormone for the production of active metabolites [11]. DHEA administration is not yet considered a medical treatment though this steroid has been demonstrated to induce specific metabolic effects [4,12,13] and to counteract the age-induced changes at the adrenal gland level as well as to increase anxiolytic substances (allopregnanolone) and some neuropeptides (i.e. β -endorphin).

β -endorphin is the most important and biologically active endogenous neuropeptide involved in several brain functions. Previous studies have demonstrated high plasma β -endorphin levels in obese subjects and that β -endorphin release is induced after an oral glucose tolerance test (OGTT) both in normal and in obese women [10]. Interestingly, when post-menopausal women with normal Body Mass Index (BMI) underwent an OGTT, they lack the ability to increase plasma circulating

β -endorphin under the glucose load since a specific modulation of the opioidergic system due to sex steroid hormone balance has been proposed to modify β -endorphin response and response to metabolic stimuli, such as glucose load [10,14–16].

We aimed to evaluate plasma β -endorphin levels in response to an OGTT in early and late, non-obese and obese postmenopausal women, before and after 6 months of oral supplementation with 50 mg/day of oral DHEA, in order to investigate if an integrative treatment with DHEA could restore/modify the control of insulin and glucose secretion as well as of β -endorphin release in response to glucose load.

Materials and methods

Subjects

Forty-six healthy postmenopausal women (age range 50–65 years) were studied. The study was performed to evaluate the effect of oral DHEA 50 mg administration in early postmenopausal women (50–55 years) both of normal weight (BMI = 20–24) (group A, $n = 12$, BMI = 22.1 ± 0.5) and overweight (BMI = 26–30) (group B, $n = 12$, BMI = 28.2 ± 0.5), and late postmenopausal women (60–65 years) both normal weight (group C, $n = 11$, BMI = 22.5 ± 0.6) and overweight (group D, $n = 11$, BMI = 27.9 ± 0.4) undergone oral glucose tolerance test.

The trial was prospective and lasted 6 months. All women were given DHEA (50 mg p.o./day) (Rottapharm, Milan, Italy) for 6 months, between 8.00 and 9.00 am. 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. 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.

Protocol

A transvaginal ultrasound examination was performed before treatment and after 3 and 6 months of treatment to evaluate the endometrial thickness. A blood specimen to measure baseline levels of circulating DHEA, DHEAS, progesterone, androstenedione, estradiol, luteinizing hormone (LH), follicle stimulating hormone (FSH), β -endorphin, insulin, and glycemia was drawn at 8.00 am, after overnight fasting and before any drug administration, at each visit.

Oral glucose tolerance test

Before and after 6 months of supplementation with 50 mg, oral DHEA patients underwent an oral glucose tolerance test (75 g) (OGTT) between 8.30 and 9.00, after overnight fasting. In order to limit the stress of the venipuncture, a polyethylene catheter inserted in an antecubital vein was kept patent by a slow infusion of saline solution. Blood specimens were collected on ice into heparinized tubes at times 0, 30, 60, 90, 120 min after glucose administration, centrifuged immediately and the plasma stored at -20° until assay. Plasma glucose, insulin, and β -endorphin levels were evaluated before and during the OGTT. Glucose and insulin levels were assayed using commercially available radioimmunoassay (RIA) kits (Radim, Rome, Italy).

β -endorphin assay

Extraction

Sep-Pak C_{18} Cartridges, previously activated with 5 ml of methanol to ameliorate the retention of the β -EP on the cartridges, were used to extract and purify the plasma samples (1 ml). Then the cartridges were washed in sequence with 5 d of bidistilled water and acetic acid 0.5 N, respectively. The last passage was filtration with 5 ml of 75% acetonitrile and 25% acetic acid. The elution extracts obtained from this passage were dried under vacuum and redissolved with a 300 μ l mixture composed of 49.8 g $Na_2 PO_4 \cdot 2H_2O$, 18 g EDTA, and 10 g bovine serum albumin dissolved in 2:1 of bidistilled water [13,14,17,18].

Radioimmunoassay

Synthetic human β -endorphin (Organon, Oss, The Netherlands) was used for standard curve and β -EP 10 pCi (Amersham International, Buckinghamshire, England) was used as a tracer. Anti-human β -endorphin 1–6 (anti- β -EP 1–210 (SigmaTau, St. Louis, MO) was used with the final concentration of 1:600,000. The sensitivity of β -endorphin radioimmunoassay ($n=12$) was 2.5 fmol/ml; water blanks gave a constant binding over 97.5%, which is equal to 0. The recovery rate ($n=14$) of labeled β -endorphin added to plasma samples subjected to extraction and chromatographic purification was 69 k 7.7%. The inter- and intra-assay coefficients of variation ($n=12$) were 9% and 6%, respectively. The results are expressed in fmol/ml.

Assays

All hormonal determinations were carried out during the same assay. Plasma LH and FSH, DHEA, DHEAS, Androstenedione (A), Progesterone (P), and Estradiol (E2) concentrations were determined using commercially available radioimmunoassays kits (Radim, Pomezia, Rome, Italy). The intra-assay and inter-assay coefficients of variation (CV) and the sensitivity of the assay were: 7.8 and 8.3%, and 0.02 ng/ml for DHEA, 6.8 and 8.5%, and 0.02 ng/ml for DHEAS, 4.2 and 7.6%, and 0.03 ng/ml for A, 6.6 and 11.7%, and 0.12 ng/ml for P, 4.6 and 8.5%, and 4.7 pg/ml for E2.

The sensitivities of the assays for LH and FSH were 0.20 mIU/ml and 0.18 mIU/ml, respectively, and the intra- and inter-assay CVs were 2.8 and 3.3 for LH, and 1.97 and 4.11 for FSH.

Statistics

The data were expressed as mean \pm SEM. Basal hormone levels, ovarian and adrenal steroids, and endometrial thickness evaluation were analyzed with a multiple analysis of variance (ANOVA) and compared by using a paired Student's *t*-test. The area under the curve (AUC) of OGTT was computed using the trapezoid formula. HOMA index was computed to estimate the sensitivity to insulin; it can be calculated by homeostasis model assessment of insulin resistance (HOMA-IR) as (fasting insulin μ U/l) \times (fasting glucose mmol/l)/22.5. The cutoff value we used is 2.71 as previously stated [19].

Results

Table 1 summarizes the hormonal patterns of all the groups of patients under study. DHEA, DHEAS, β -endorphin, and A plasma levels were lower in baseline conditions in older women (Groups C and D) than younger women (Group A and B). LH plasma levels were lower in older obese women (Group D) than younger patients (Group A and B).

After 3 and 6 months of DHEA administration, there was a significant decrease of LH, FSH, and HOMA index while DHEA, DHEAS, β -endorphin, E2 and A plasma levels were significantly increased in comparison to basal values (Table 1). When OGTT was performed, the dynamics of insulin response to oral glucose load showed some changes. In fact, AUC of insulin, though not reaching the statistical significance, showed a reduction in normal weight early and late postmenopausal women (Group A and Group C) as well as in overweight early and late postmenopausal women (Group B and Group D) after 3 and 6 months of treatment (Figure 1). The analysis of the AUC of glycemia showed no dynamics changes in response to an oral glucose load (Figure 1). When considering the AUC of β -endorphin response to OGTT, all groups showed a progressive significant increase after 3 and also after 6 months of treatment in comparison to baseline and 3 months of treatment (Figure 1), reinforcing the observed result of β -endorphin plasma levels increase as previously described (Table 1).

Discussion

The present study demonstrated that DHEA supplementation determines the restoration of β -endorphin response to OGTT normally disappeared after menopause. Moreover, it confirms the significant effects of DHEA supplementation on the increase

of circulating levels of β -endorphin in different groups of postmenopausal women.

During the menopausal transition, there is a reduced β -endorphin level due to the hypoestrogenic and it is completely reversed by the administration of hormonal replacement therapy [14,17,18,20,21]. Stomati et al. reported a significant and progressive increase in plasmatic β -endorphin concentrations in postmenopausal women treated for three months with DHEA at the dose of 50 mg/day [15]. Later, Genazzani et al. have described an increase in β -endorphin levels in healthy postmenopausal women, in early and late postmenopausal women and aging males with partial androgen deficiency, respectively treated with 12 months DHEA therapy at the dose of 10 mg/day, 25 mg/day, and 25 mg/day, respectively [20,21].

The present data indicate a putative primary role of sex steroids derived from DHEA conversion or DHEA itself in controlling β -endorphin synthesis and/or release, suggesting that the specific modulation of the pituitary opioidergic system due to adrenal steroid hormone balance, could modify β -endorphin release and response to metabolic stimuli, such as glucose load.

Even if our interesting results confirmed an increase in plasma β -endorphin levels under DHEA treatment both in basal and in dynamic conditions, the identification of the real mechanisms of DHEA regulation at the basis of β -endorphin increase is still poorly understood. β -endorphin and ACTH synthesis and release are modulated by several neurotransmitters such as noradrenaline, dopamine, serotonin, acetylcholine, GABA all acting on hypothalamic CRF release [14,22,23].

Table 1. Hormonal characteristics of patients under study.

Patients	LH mUI/ml	FSH mUI/ml	DHEA ng/dL	DHEAS g/mL	Estradiol pg/mL	β -endorphin pg/mL	P ng/mL	A ng/dL	Insulin μ U/ml	Glucose mg/dl	HOMA index
Baseline											
Group A	34.8 \pm 3.0	64.9 \pm 4.5	4.0 \pm 0.2	0.7 \pm 0.04	18.6 \pm 1.3	15.0 \pm 3.5	0.3 \pm 0.05	1.6 \pm 0.2	9.4 \pm 0.3	90.7 \pm 2.5	3.0 \pm 0.8
Group B	34.3 \pm 3.0	63.8 \pm 5.4	3.8 \pm 0.4	0.6 \pm 0.09	19.7 \pm 2.4	19.0 \pm 3.0	0.3 \pm 0.06	1.5 \pm 0.2	10.6 \pm 0.5	91.3 \pm 1.9	3.6 \pm 0.5
Group C	32.4 \pm 3.5	71.8 \pm 6.8	3.0 \pm 0.2 ^a	0.4 \pm 0.08 ^a	16.0 \pm 1.5	8.5 \pm 2.5 ^a	0.3 \pm 0.06	0.6 \pm 0.1 ^a	9.9 \pm 0.4	91.6 \pm 1.5	2.8 \pm 0.9
Group D	24.3 \pm 3.5 ^a	51.8 \pm 9.9	3.0 \pm 0.3 ^a	0.4 \pm 0.07 ^a	17.5 \pm 1.6	9.0 \pm 2.5 ^a	0.3 \pm 0.05	0.8 \pm 0.1 ^a	11.4 \pm 0.6	90.9 \pm 2	3.9 \pm 0.4
3rd month of treatment											
Group A	27.5 \pm 3.5 ^b	49.0 \pm 4.5 ^b	11.7 \pm 1.0 ^b	2.6 \pm 0.1 ^b	67.5 \pm 4.3 ^b	30 \pm 4.6 ^b	0.4 \pm 0.06	3.2 \pm 0.3 ^b	9.0 \pm 0.2	88.9 \pm 1.9	2.6 \pm 0.3 ^b
Group B	30.4 \pm 3.8 ^b	56.5 \pm 7.1 ^b	13.1 \pm 0.9 ^b	2.6 \pm 0.2 ^b	54.6 \pm 3.8 ^b	32 \pm 5.0 ^b	0.4 \pm 0.07	3.5 \pm 0.4 ^b	11.4 \pm 0.6	90.6 \pm 2.1	3.2 \pm 0.8 ^b
Group C	23.1 \pm 7.4 ^b	54.3 \pm 7.7 ^b	10.6 \pm 1.0 ^b	1.5 \pm 0.1 ^b	57.8 \pm 2.9 ^b	15 \pm 2.7 ^b	0.4 \pm 0.08	2.8 \pm 0.3 ^b	10.4 \pm 0.5	90.3 \pm 1.9	2.5 \pm 0.4 ^b
Group D	13.7 \pm 3.1 ^b	44.5 \pm 5.4 ^b	10.7 \pm 0.9 ^b	1.5 \pm 1.2 ^b	78.0 \pm 9.7 ^b	16 \pm 3.1 ^b	0.3 \pm 0.05	3.2 \pm 0.3 ^b	12.2 \pm 0.5	89.2 \pm 2.2	3.4 \pm 0.3 ^b
6th month of treatment											
Group A	21.5 \pm 2.0 ^b	36.8 \pm 3.5 ^b	12.2 \pm 0.4 ^b	2.9 \pm 0.2 ^c	72.6 \pm 3.0 ^b	34 \pm 3.2 ^b	0.4 \pm 0.07	3.9 \pm 0.2 ^b	9.1 \pm 0.5	89.2 \pm 2	2.3 \pm 0.8 ^c
Group B	24.5 \pm 3.0 ^b	40.0 \pm 4.2 ^b	13.6 \pm 0.9 ^b	2.7 \pm 0.2 ^c	66.8 \pm 4.7 ^b	36 \pm 1.1 ^b	0.4 \pm 0.07	4.3 \pm 0.3 ^b	10.6 \pm 0.5	90.1 \pm 2.3	2.9 \pm 0.8 ^c
Group C	19.2 \pm 5.3 ^b	38.1 \pm 4.8 ^b	11.7 \pm 1.0 ^b	2.2 \pm 0.1 ^c	63.1 \pm 3.9 ^b	20 \pm 1.8 ^b	0.4 \pm 0.08	4.3 \pm 0.5 ^b	10.8 \pm 0.7	91.2 \pm 2.2	2.4 \pm 0.8 ^c
Group D	11.6 \pm 2.7 ^b	34.1 \pm 3.6 ^b	12.1 \pm 0.5 ^b	2.0 \pm 0.1 ^c	94.3 \pm 11.3 ^b	20 \pm 1.5 ^b	0.4 \pm 0.03	3.9 \pm 0.1 ^b	11.2 \pm 0.3	90.5 \pm 1.6	2.9 \pm 0.5 ^c

Mean \pm SEM.

^a p < .05 vs. groups A and B.

^b p < .05 vs. basal conditions.

^c p < .01 vs. basal conditions.

LH: luteinizing hormone; FSH: follicle stimulating hormone; DHEA/DHEAS: dehydroepiandrosterone/dehydroepiandrosterone sulfate.

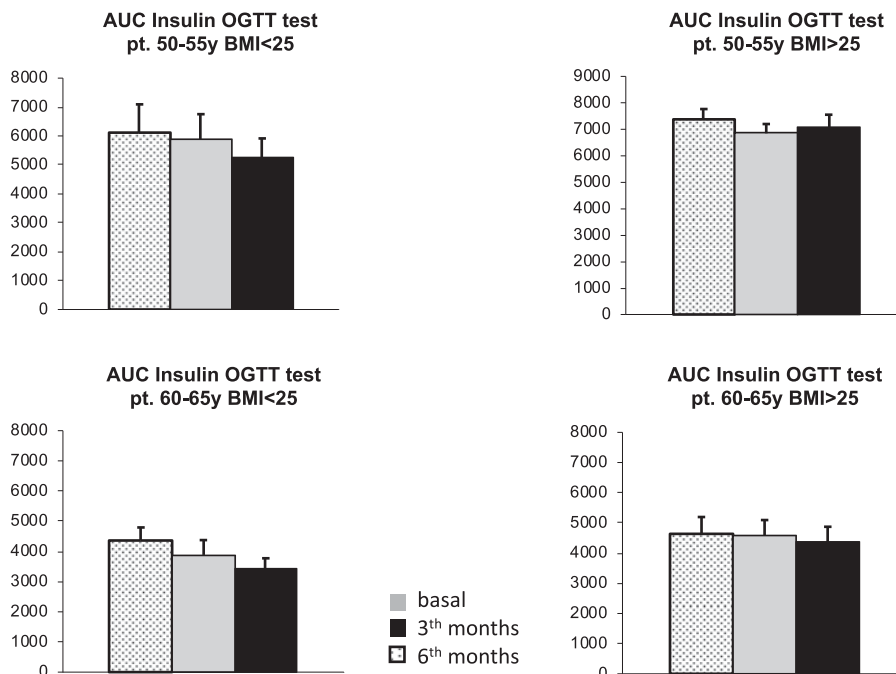


Figure 1. Analysis of the Area Under the Curve (AUC) of Insulin, Glycemia and β -endorphin in normal-weight and over-weight, early and late post-menopausal women in response to oral glucose load.

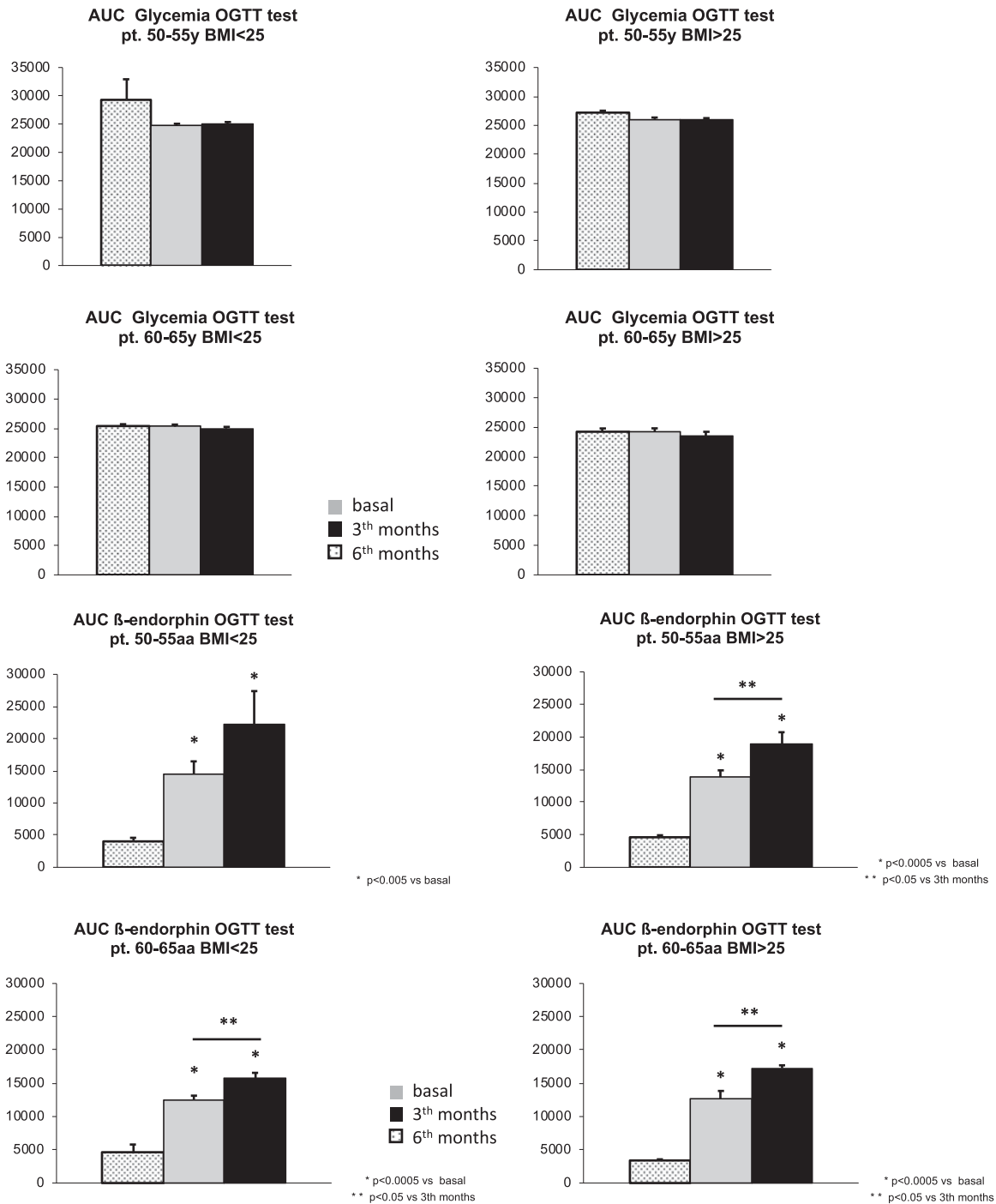


Figure 1. Continued

Previous studies investigating β -endorphin in obese menstruating women or the state of the post-menopause in women with normal body mass index, demonstrated that, in these subjects, the release of the pro-opiomelanocortin (POMC)-related peptides is decreased during postmenopause. It is plausible to speculate that the rise in β -endorphin plasma levels may be the effect of direct activation of this neuroendocrine pathway due to the restoration of a steroidogenic milieu after 3 months of DHEA supplementation. Hence, according to the improved AUC of β -endorphin in response to OGTT, all groups showed a progressive significant increase after 3 and also after 6 months of

treatment in comparison to baseline and 3 months of treatment, respectively. In our opinion, the rise of β -endorphin, androgens together with the reduction of circulating levels of cortisol despite minimal variations of glycemia and insulinemia, may be suggestive of a DHEA-induced 'younger adrenal setting' probably due to the restoration of adrenal enzymatic environment which is similar to those observed in younger women. We can infer that the restitution of a newer β -endorphin response to OGTT after DHEA supplementation and the consequent improvement of the steroidal environment may stimulate CRF and POMC re-arranging a new equilibrium so that the new dynamic of the

enzymes expression/synthesis is able to codify for β -endorphin release.

In addition to the effects at the central nervous system (CNS) level, it has to be pointed out that DHEA administration reduced HOMA index in all subjects, independently from BMI and age, thus supporting the hypothesis of the relevant effect(s) of DHEA and of its metabolites on insulin sensitivity [11,24].

The withdrawal of sex steroids in the climacteric period causes a decrease in β -endorphin levels, due to an impairment of the central neuroendocrine system controlling β -endorphin release [17,25].

The main limitations of our study are the small sample groups and the lack of a randomized control group.

In fact, even if acquired data derived from a small subgroup of patients neglecting somewhat from conclusive scientific evidence; this limited experience adds novel contents about the relations between DHEA and metabolic profile in aging women.

However, the lack of definitive evidence for biological mechanisms of DHEA as integrative treatment and of metabolic effects in postmenopausal women might encourage further studies.

Disclosure statement

No potential conflict of interest was reported by the authors.

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