CLINICAL STUDY

Metabolic effects of dehydroepiandrosterone replacement therapy in postmenopausal women

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Abstract

Objective: To investigate whether long-term treatment with dehydroepiandrosterone (DHEA) in postmenopausal women can modify insulin sensitivity and plasma lipid profile.

Design and methods: Twenty healthy postmenopausal women with serum dehydroepiandrosterone sulfate (DHEA-S) concentrations $<2.5 \mu\text{mol/l}$ were enrolled and randomly assigned to two different treatment groups: group 1 were treated with micronized DHEA, 25 mg/day at 0800 h for 12 months; group 2 were treated with an identical placebo tablet. At the beginning and at the end of the study, plasma lipid profile, glucose tolerance (oral glucose tolerance test) and insulin sensitivity (euglycemic hyperinsulinemic clamp: M index) were assessed.

Results: After 12 months, the group treated with DHEA showed a considerable improvement of insulin sensitivity (M index $+29.55\%$, $P = 0.01$) and lipid pattern (high-density lipoprotein cholesterol $+11.61\%$, $P = 0.03$; low-density lipoprotein cholesterol -11.07% , $P = 0.04$; triglycerides -19.60% , $P = 0.03$), but glucose tolerance did not change. No modifications were observed in the placebo group.

Conclusions: Long-term treatment with DHEA ameliorates some metabolic parameters that are linked to increased cardiovascular risk and, consequently, this seems to be an interesting therapeutic tool in the management of the postmenopausal syndrome.

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Introduction

Plasma dehydroepiandrosterone sulfate (DHEA-S), which originates from and is converted to dehydroepiandrosterone (DHEA) in peripheral tissues, is the major secretory steroid of the adrenal gland. Its secretion is stimulated by corticotropin (1), levels of which may increase with age (2). However, both cross-sectional (3–6) and longitudinal (7) data have clearly indicated that plasma concentrations of DHEA-S decrease with age. Adrenal secretion of DHEA-S increases during adrenarche in children at the age of 6–8 years, and maximal values of circulating DHEA-S are reached between the ages of 20–30 years. Thereafter, serum DHEA-S concentrations decrease progressively at a relatively constant rate of 2% per year. The up to 95% reduction in the secretion of DHEA-S by the adrenals during aging results in a dramatic reduction in the formation of androgens and estrogens in peripheral target tissue – a situation that has been suggested to be associated with age-related diseases, including cardiovascular diseases (8–10). The role of DHEA in glucose and lipid metabolism is not clear. Although there is no relationship between DHEA-S

concentrations and glucose tolerance in men, the relationship between DHEA-S, lipids and lipoproteins is controversial, with some studies showing a less atherogenic lipid and lipoprotein profile (11–13) and other studies showing no association (14) or increased low-density lipoprotein (LDL) cholesterol (10). Insulin concentrations are negatively correlated with DHEA-S in premenopausal women (15) and acute hyperinsulinemia significantly reduces DHEA-S plasma concentrations in healthy men (16). Nestler *et al.* (17) have recently proposed that the insulin-induced suppression of DHEA and DHEA-S may provide the ‘missing link’ between hyperinsulinemia and atherosclerosis. Many pieces of evidence have clearly demonstrated an association between these two last conditions and it is well accepted that the influence of hyperinsulinemia on atherosclerosis is mediated by the existing insulin resistance (18).

Concerning DHEA-S and insulin resistance, however, there are conflicting results. Observational studies in insulin-resistant conditions such as age (19), essential hypertension (20) and female hyperandrogenism (21) clearly demonstrate an association between low DHEA-S plasma concentrations and reduced insulin

sensitivity. Intervention studies with DHEA supplementation, however, have given conflicting results: improvement of insulin sensitivity in experimental animals (22); no modification of insulin sensitivity in polycystic ovary syndrome (PCOS) with short-term infusion (23), in normal individuals undergoing short-term administration (24), in obese adolescents (25) and adults (26) with medium-term treatment and in postmenopausal women after long-term substitution (27); amelioration of insulin resistance in a case of diabetes and hyperandrogenism (28). Moreover, treatment of PCOS with an insulin-sensitizer drug both improved insulin resistance and reduced DHEA-S plasma concentrations (29).

As no data are available from long-term studies concerning glucose and insulin metabolism in postmenopausal women during DHEA replacement therapy, the specific aim of our study was to determine whether and to what extent long-term treatment with DHEA can modify insulin sensitivity, assessed with the reference method of the glucose clamp technique. A second specific aim was to assess whether long-term DHEA treatment affects other major cardiovascular risk factors including plasma concentrations of total cholesterol, high-density lipoprotein (HDL) and LDL cholesterol and triglycerides, and body mass index (BMI).

Materials and methods

Participants

For this study, 20 healthy adrenal-androgen-deficient postmenopausal women (serum DHEA-S concentrations $<2.5 \mu\text{mol/l}$), who had never been treated with hormonal replacement therapy and who gave their informed consent, were enrolled and randomly assigned to two different treatment groups: group 1 (10 women: mean age 57.6 ± 4.5 years, mean duration of amenorrhea 11.3 ± 3.7 years, BMI $23.7 \pm 3.2 \text{ kg/m}^2$) were treated with oral DHEA, 25 mg/day at 0800 h for 12 months. DHEA was provided as tablets of a micronized galenic compound, prepared by a local chemist's shop. In group 2 (10 women, mean age 55.1 ± 3.8 years, mean duration of amenorrhea 10.3 ± 3.1 years, BMI $23.9 \pm 3.1 \text{ kg/m}^2$), patients received an identical placebo tablet. This dose of DHEA was chosen because it effectively brings serum DHEA-S concentrations into the premenopausal range, without the risk of hyperandrogenism or other side effects (30, 31). Study drug and placebo were given to each patient in boxes containing 90 tablets – the supply for a 3-month period. To check compliance with the medication tablets, patients were asked to bring these boxes at each 3-month control visit, when the remaining tablets were counted. During these control visits, a complete clinical examination and routine laboratory tests, including a side effects or adverse events check, were

performed. Before each woman was included in the study, acute or chronic illnesses were excluded by means of clinical examination and routine laboratory investigation. Exclusion criteria also included history of diabetes mellitus and other metabolic or endocrine disorders. No specific diet instructions were given to the study participants.

The study procedure was approved by the Ethics Committee of the University of Messina School of Medicine.

Methods

At the beginning and end of the study period, glucose tolerance during an oral glucose tolerance test (OGTT; 75 g) was evaluated in each woman, according to WHO criteria (32). Insulin sensitivity was assessed by means of the euglycemic hyperinsulinemic clamp technique, according to DeFronzo *et al.* (33) as previously described in detail (34). Briefly, in each participant, after an overnight fast, a 2-h euglycemic hyperinsulinemia was established with a primed constant infusion of regular insulin. To suppress hepatic glucose production, the target clamped insulin concentration was $70\text{--}80 \mu\text{U/ml}$, achieved with an infusion rate of 1 mU/kg body weight per min. Basal glucose concentrations were maintained by means of a variable 20% glucose infusion. Plasma glucose was sampled every 10 min and measured with a Beckman Glucose Analyzer 2 (Beckman Instruments, Milan, Italy). The coefficient of variation for plasma glucose was always $<5\%$ during the last 60 min of the clamp. The glucose infusion rate required to maintain euglycemia, expressed as mg/kg per min and calculated during the second hour of the clamp, was used as insulin sensitivity index (M index).

Fasting plasma concentrations of total cholesterol, triglycerides (enzymatic methods, Roche Diagnostics, Milan, Italy) and HDL cholesterol (after plasma precipitation with dextran–magnesium) were also measured. LDL cholesterol was estimated indirectly by means of the Friedewald formula (35).

Finally, plasma concentrations of DHEA-S and testosterone were also estimated by solid-phase immunoassays (Roche Diagnostics, Milan, Italy) at the beginning and end of the study. The lowest detectable values were $0.003 \mu\text{mol/l}$ for DHEA-S and 0.069 nmol/l for testosterone plasma concentrations; intra- and interassay coefficients of variation were 2.8% and 3.6% respectively for DHEA-S and 1.4% and 2.2% respectively for testosterone.

Statistical analysis

Data are expressed as mean $\pm$ S.D. Results were analyzed using the paired Student's *t*-test and Pearson's correlation coefficient. A two-tailed *P* value <0.05 was considered significant.

Results

Baseline and 12-month values of M index, total cholesterol, HDL and LDL cholesterol, triglycerides, BMI, DHEA-S and testosterone in both groups of postmenopausal women are reported in Table 1. Baseline values were not different in the two groups. In group 1 (active treatment), the M index significantly increased after 12 months with respect to the pretreatment value, whereas it did not change in group 2 (placebo treatment). In group 1 there was also a significant reduction in plasma triglyceride and LDL cholesterol concentrations, with a concomitant increase in HDL cholesterol and no modification of total cholesterol at the end of the study. No differences in plasma lipid concentrations were observed in group 2. BMI did not significantly change from baseline to the end of the study in either group. Serum concentrations of DHEA-S increased significantly, but those of testosterone had not changed at the end of the study in group 1. No modifications of hormone concentrations were observed in group 2. Glucose tolerance during the OGTT was always normal at baseline and had not changed in any of the women at the end of the study. No correlations were found between DHEA-S concentrations and metabolic parameters in the two groups, at either the beginning or the end of the study. No relevant side effects were recorded during the study period; only one patient developed slight acne at the beginning of treatment and it disappeared spontaneously later. The compliance of each patient with treatment was apparently high, as the number of the unused pills given back at each visit was never greater than 8 – that is, <10% of the original supplies (mean $\pm$ s.d.: 3 ± 2 pills).

Discussion

Our results show that low-dose, long-term DHEA replacement therapy in postmenopausal women significantly improves some metabolic parameters that are linked to an increased cardiovascular risk. After 12 months of oral micronized DHEA, insulin sensitivity

increased and plasma lipid concentrations, although normal at baseline, became significantly less atherogenic (increased HDL cholesterol, reduced LDL cholesterol and triglycerides).

Concerning DHEA replacement and insulin resistance, our results differ from those obtained during short-term or medium-term treatment by others. Schriock *et al.* (23) demonstrated that a 17-h DHEA infusion did not modify insulin sensitivity indices, indirectly evaluated from OGTT parameters, in women with PCOS and in obese women. Vogiatzi *et al.* (25) treated a small group of morbidly obese adolescents with 40 mg DHEA twice daily for 8 weeks, and did not observe modification of insulin sensitivity indices. Similar results were reported by Usiskin *et al.* (26) in obese men and by Casson *et al.* (30) in postmenopausal women.

In this study we selected a group of postmenopausal women with clearly demonstrated DHEA deficiency (plasma DHEA-S <2.5 μ mol/l) and treated them for a long period (12 months) with a low dose of DHEA-S. Moreover, insulin sensitivity was assessed using the 'gold standard' method of the euglycemic hyperinsulinemic clamp. These main characteristics of our study could explain the differences between our results and those of others.

We have no evidence concerning the mechanisms by which DHEA can improve insulin sensitivity in postmenopausal women; however, a post-receptor effect of DHEA has been clearly demonstrated after even a short-term infusion (23). This effect, which is probably not of sufficient duration in short-term observations, could well be effective on insulin sensitivity in long-term treatment such as ours.

The improvement in plasma lipid profile that we also observed is probably a consequence of the improved insulin sensitivity. Previous reports on DHEA-S and plasma lipid profile have indicated that DHEA concentrations are negatively correlated with those of total cholesterol (11) and LDL cholesterol (13), and positively associated with HDL cholesterol (12). Intervention studies confirm that DHEA administration lowers total and LDL cholesterol (24), but they suggest

Table 1 Mean ($\pm$ s.d.) values of metabolic and hormonal parameters at the beginning (baseline) and at the end (12 months) of the study, in two groups of postmenopausal women (group 1: DHEA-treated; group 2: placebo-treated).

	Group 1			Group 2		
	Baseline	12 months	P	Baseline	12 months	P
M index (mg/kg per min)	6.7 $\pm$ 1.5	8.68 $\pm$ 1.8	0.01	6.4 $\pm$ 1.2	6.3 $\pm$ 0.8	NS
Total cholesterol (mmol/l)	5.26 $\pm$ 0.74	4.99 $\pm$ 0.70	NS	5.31 $\pm$ 0.75	5.37 $\pm$ 0.71	NS
HDL cholesterol (mmol/l)	1.55 $\pm$ 0.16	1.73 $\pm$ 0.19	0.03	1.52 $\pm$ 0.18	1.51 $\pm$ 0.40	NS
LDL cholesterol (mmol/l)	3.25 $\pm$ 0.31	2.89 $\pm$ 0.42	0.04	3.37 $\pm$ 0.35	3.45 $\pm$ 0.69	NS
Triglycerides (mmol/l)	1.02 $\pm$ 0.23	0.82 $\pm$ 0.15	0.03	0.94 $\pm$ 0.19	0.92 $\pm$ 0.16	NS
BMI (kg/m ²)	23.7 $\pm$ 3.2	23.1 $\pm$ 2.2	NS	23.9 $\pm$ 3.1	24.1 $\pm$ 2.7	NS
DHEA-S (μ mol/l)	1.38 $\pm$ 0.29	1.92 $\pm$ 0.51	0.009	1.36 $\pm$ 0.32	1.37 $\pm$ 0.24	NS
Testosterone (nmol/l)	1.38 $\pm$ 0.79	1.31 $\pm$ 0.45	NS	1.39 $\pm$ 0.86	1.35 $\pm$ 0.52	NS

that it can slightly reduce HDL cholesterol concentrations in healthy older men (13) and in perimenopausal (27) or postmenopausal (30) women. To appreciate better the effect of replacement therapy, in our study we selected women with an evident deficiency of DHEA-S production and we prolonged the treatment for 12 months with a low, not androgenizing, dose of DHEA. This different methodological approach could explain the positive results we obtained in terms of plasma lipid profile (reduced LDL cholesterol and triglycerides, increased HDL cholesterol), probably as a consequence of the increased sensitivity to insulin.

A possible limitation of our study is represented by the lack of an intermediate metabolic evaluation; as far as compliance was concerned, however, pill counting led us to believe that it was high.

In conclusion, in a group of healthy postmenopausal women low-dose, long-term treatment with DHEA significantly improved insulin resistance and plasma lipid profiles. The beneficial effects on these cardiovascular risk factors may suggest a role for DHEA replacement therapy, alone or in association with other substitution treatments (36), in conditions of risk for cardiovascular events, such as the menopause. Only long-term randomized trials with clinically relevant end-points can confirm this possibility.

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