Effects of Transdermal Application of 7-oxo-DHEA on the Levels of Steroid Hormones, Gonadotropins and Lipids in Healthy Men

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Summary

The aim of this study was to investigate the effect of 7-oxo-DHEA (dehydroepiandrosterone) on the serum levels of steroid sexual hormones, gonadotropins, lipids and lipoproteins in men. 7-oxo-DHEA was applied onto the skin as a gel to 10 volunteers aged 27 to 72 years for 5 consecutive days. The single dose contained 25 mg 7-oxo-DHEA. Serum concentrations of testosterone, estradiol, cortisol, androstenedione, luteinizing hormone (LH), follicle-stimulating hormone (FSH), sex hormone binding globulin (SHBG), total cholesterol, HDL- and LDL-cholesterol, triglycerides, apolipoprotein A-I and B and lipoprotein(a) were measured before the beginning and shortly after the end of the steroid application. After the treatment, we noted the following significant changes: a decline of testosterone and estradiol levels, increase of LH, HDL-cholesterol and apolipoprotein A-I levels. The decrease of total cholesterol levels was of the borderline significance. A slight but significant increase was found in apolipoprotein B and lipoprotein(a). The most expressive was the fall of the atherogenic index. We suggest that the gel containing 7-oxo-DHEA might be a suitable drug for improving the composition of the steroid and lipid parameters in elderly men.

Introduction

Dehydroepiandrosterone (DHEA) and especially its sulfate (DHEAS) are the most abundant secretory products of human adrenal glands (Migeon *et al.* 1957, Šonka 1976). DHEA is known to be a precursor of the sex hormones testosterone and estradiol, but it exerts several additional physiological effects (Lardy *et al.* 1995). Its relatively large doses decrease blood cholesterol levels in men (Nestler *et al.* 1988). Exogenous DHEA induces the activity of mitochondrial and cytosolic thermogenic enzymes in rat liver (Lardy *et al.* 1995). Animal as well as human tissues convert DHEA to its 7α-, 7β-hydroxylated and 7-oxo derivatives *in vitro* (Hampl *et al.* 1997). Some 7-oxygenated steroids are even more active than DHEA itself regarding the induction of thermogenic enzymes (Lardy *et al.* 1995).

Since Baulieu (1996) introduced the term "fountain of youth" for DHEA, a number of hypotheses emerged about its protective role in coronary heart

disease (Poršová-Dutoit *et al.* 2000). However, the results of the studies concerning DHEA(S) protective effect on coronary artery diseases are controversial (Poršová-Dutoit *et al.* 2000). Recently, we examined the effects of short-term transdermal application of DHEA on the levels of steroid and protein hormones and on the lipid parameters in healthy men (Šulcová *et al.* 2000). We found several changes in serum steroid and gonadotropin patterns, but the levels of lipids and lipoproteins – total cholesterol (TC), high- and low-density lipoprotein cholesterol (HDL-C and LDL-C), triglycerides (TG), apolipoprotein A-I (Apo A-I), apolipoprotein B (Apo B) and lipoprotein(a) [Lp(a)] remained almost unchanged.

There is evidence that 7-oxygenated derivatives of DHEA are probably mediators of the beneficial effects of DHEA and that their activity as immunomodulators and metabolic mediators is higher than that of the maternal compound. It is also of interest that skin is an effective site of 7-hydroxylation of DHEA (Šulcová *et al.* 1968, Faredin *et al.* 1969). This fact might explain the effectiveness of DHEA application by transdermal route (Labrie *et al.* 1996).

We were hence interested whether 7-oxygenated DHEA after transdermal application in healthy men would produce alterations (especially favorable) in their lipid spectra. Therefore, 7-oxo-dehydroepiandrosterone was administered as a gel for 5 consecutive days to 10 male volunteers of different ages. The fasting serum levels of selected steroids, proteohormones, lipids and lipoproteins were measured before and after application.

Methods

Steroids and chemicals

3β-hydroxy-5-androstene-7,17-dione (7-oxo-DHEA) was from Steraloids Inc. (Wilton, NH, USA). Diethyl ether and chemicals used for radioimmunoassay, all of analytical grade, were purchased from Merck (Darmstadt, Germany).

Subjects

The group of volunteers consisted of 10 informed healthy men 29-72 (50.5 ± 13.7) years old (mean ± S.D.). They were neither on regular medication nor had health risks except for the higher age of some of them.

Treatment protocols

7-oxo-dehydroepiandrosterone was given transdermally as a gel containing 0.5 g 7-oxo-DHEA per 100 g (A. Nováček, AREKO, Prague, CR). Approximately 5 g of the gel, corresponding to a daily dose of 25 mg 7-oxo-DHEA, were applied onto a 200 cm^2 abdominal skin area before sleeping at 22:00 h, for five consecutive days. Morning blood collections were performed after a night fast before the start of treatment (Day 0), and on the day after the last application (Day 6).

Table 1. The levels of steroids, gonadotropins and SHBG before and after 5 days of transdermal treatment by 7-oxo-DHEA in 10 healthy men of various ages.

Substance	Unit	Before application	After application	Change (%)	p<
Testosterone	nmol/l	19.3±1.6 (18.7)	16.5±1.1 (16.1)	−11.9	0.05
FTI		96.4±6.6 (95.3)	76.7±10.6 (69.9)		0.0006
Estradiol	pmol/l	2600±484 (2150)	649±65 (730)	−65.6	0.0005
Androstenedione	nmol/l	7.07±0.40 (6.84)	7.88±0.54 (7.61)	12.6	NS
Cortisol	nmol/l	675±57 (647)	607±46 (629)	−7.4	NS (p<0.076)
LH	IU/l	1.05±0.12 (1.10)	2.53±0.34 (2.45)	189.9	0.002
FSH	IU/l	4.11±0.94 (4.00)	4.35±0.61 (4.45)	11.8	NS
SHBG	nmol/l	21.2±2.5 (21.8)	24.9±3.5 (23.6)	15.4	NS

*FTI – free testosterone index was calculated as a ratio: 100 * (testosterone/SHBG). Data are given as means ± S.E.M. (median).*

Steroid determination

Serum estradiol was determined by commercial RIA kits from Immunotech (Marseille, France and Prague, Czech Republic). Testosterone (Hampl 1994), androstenedione (Putz *et al.* 1982) and cortisol (Bičíková *et al.* 1988) were assessed by RIA using antisera prepared in our laboratory and radioactive tracers (either commercial or radioiodinated in our laboratory).

Determination of proteohormones

RIA kits from Huma-Lab (Košice, Slovakia) were used for determination of LH and FSH; sex hormone-binding globulin was measured by IRMA kit from Orion Diagnostica (Finland).

Determination of lipids

Serum total cholesterol, triglycerides and HDL-C levels were measured enzymatically using kits purchased from Roche Diagnostics. LDL-C was calculated by Friedewald´s formula (serum TG values were lower than 4.5 mmol/l in the present study), serum Apo A-I, Apo B and Lp(a) assessment was performed according to the Laurent´s rocket method using Behring antisera.

Statistical analysis

For the evaluation of differences between the initial and final stage of the experiment in the studied parameters, Wilcoxon´s robust paired test was used. The mutual relations between the parameters studied were evaluated using robust Sperman´s correlations.

Results

Hormonal profiles

The differences between basal levels and those measured immediately after the treatment had been discontinued (10 h after the last dose of 7-oxo-DHEA) are demonstrated in Table 1. Thus, a very variable hormonal response was obtained after the application. Levels of sex steroids, testosterone and estradiol, decreased significantly. LH levels increased significantly, but the increase of androstenedione, SHBG and FSH was not significant. Decreasing tendency close to statistical significance was observed in cortisol.

Lipid parameters

The individual differences in lipid levels between Day 0 and Day 6 are shown in Figure 1. We found that many significant changes of lipid parameters occurred following 7-oxo-DHEA treatment. Total cholesterol was reduced, but the decrement was only of the borderline significance. HDL-C and Apo A-I significantly increased at the end of the test period. The atherogenic index (AI, calculated as ratio TC/HDL-C) decreased and this change was strongly significant. TG and LDL-C were diminished non-significantly, Apo B and Lp(a) rose slightly, but significantly.

Correlation between hormones and lipids

The age-adjusted multivariate analysis and resulting correlation of all parameters of interest at Day 0 (before treatment) are shown in Table 2. Table 3 demonstrates the same correlation coefficients of selected items summarized for both stages of the trial, i.e. Day 0 and Day 6 (before and after treatment with 7-oxo-DHEA, respectively).

Steroids. Out of all the steroids measured, cortisol only correlated positively with androstenedione before and after treatment. Testosterone correlated positively with SHBG in both stages of the trial. The only correlation at the beginning of the trial was observed between testosterone and atherogenic index (negative) and between testosterone and triglycerides (negative). The negative correlation estradiol/LH and positive correlation androstenedione /Lp(a) was found after treatment.

Proteohormones. FSH correlated positively with SHBG during both stages of the study. Negative correlation of LH with cholesterol, TG and AI, respectively, were observed before treatment. After application of 7-oxo-DHEA, a negative correlation was found between LH and AI and a positive one between HDL-C and Apo A-I. On the contrary, FSH correlated positively with LDL-C after treatment.

Lipids. In all the lipids investigated, strong mutual association of many parameters may be seen. Correlation coefficients, positive or negative, mostly reflect physiological roles of individual lipid parameters and their mutual relationship. Again, more expressed multiple correlation appeared only after the 7-oxo-DHEA challenge (TC/LDL-C, TC/Apo A-I, HDL-C/Apo A-I, HDL-C/Apo B, LDL-C/Apo A-I, LDL-C/Apo B, AI with Apo A-I and Apo B etc).
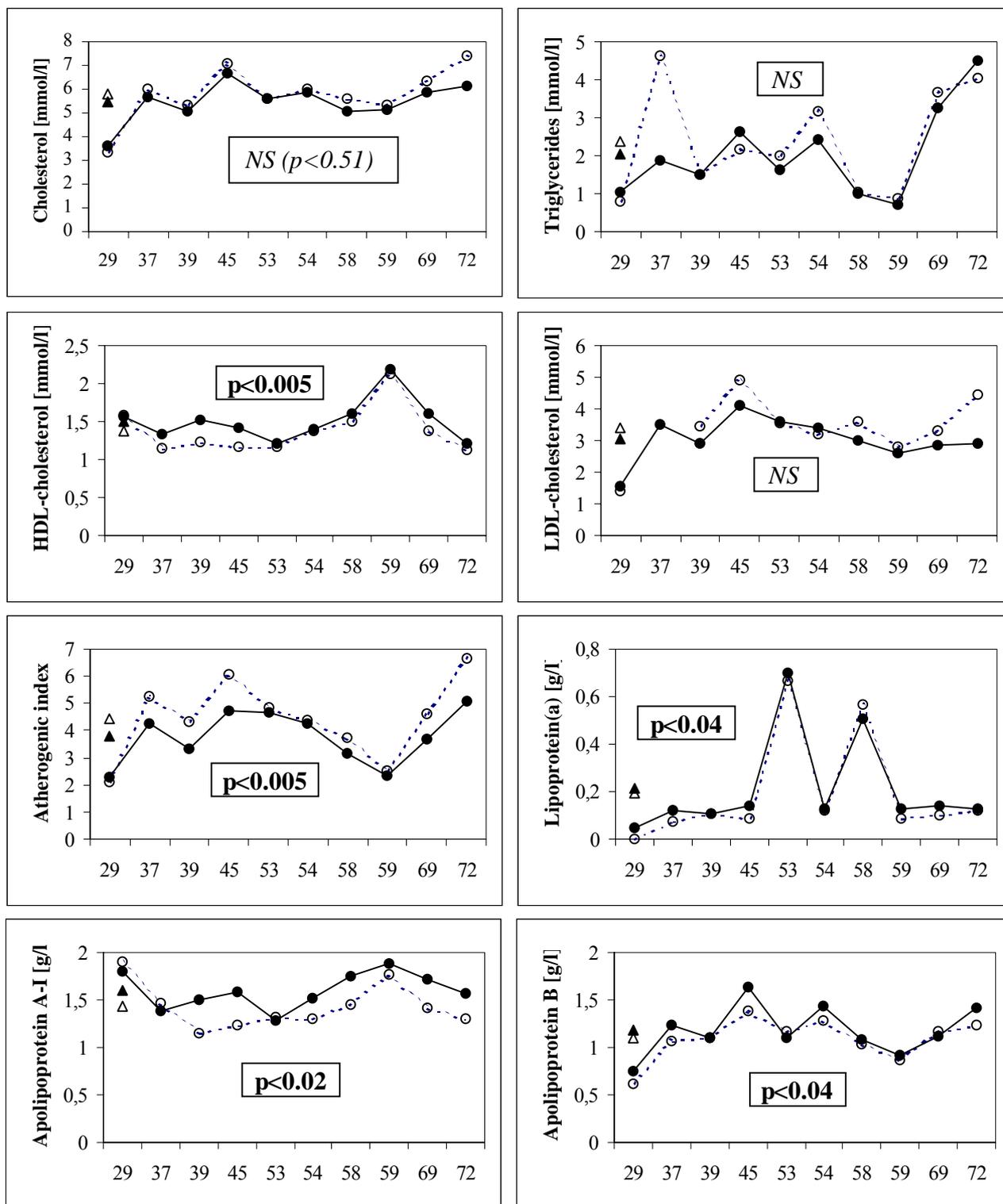
Fig. 1. *Individual levels of lipid parameters in male volunteers before and after 5 days of transdermal application of 7-oxo-DHEA. Open circles, dotted lines = Day 0 (before treatment); full dots, full lines = Day 6 (10 h after the last dose). Triangles express the mean values: open = before treatment, full = after treatment. The age of subjects is on the ordinate. The results of statistical evaluation of the differences between Days 0 and 6 (performed by Student`s paired t-test) are given in the frames.*

Table 2. Age-adjusted Spearman's correlations between the parameters investigated at the beginning of the trial.

	Steroids				Proteohormones			Lipid parameters							
	Cortisol	Testo	E-diol	A-dione	LH	FSH	SHBG	TC	HDL-C	LDL-C	AI	TG	Apo A-I	Apo B	Lp(a)
Cortisol				**0.740**											
Testo							0.793				−0.679	−0.681			
E-diol															
A-dione	0.023														
LH								−0.827			−0.747	−0.761			
FSH							0.738								
SHBG	0.011					0.023									
TC					0.006				−0.806		0.928	0.849		0.811	
HDL-C								0.009		−0.782	−0.948	−0.810			
LDL-C									0.022		0.756				
AI	0.044				0.021			0.000	0.000	0.030		0.833		0.752	
TG	0.043				0.017			0.004	0.008		0.005				
Apo A-I														−0.804	
Apo B								0.008			0.020		0.009		
Lp(a)															

The numbers above and below the diagonal represent correlation coefficients and their significance, respectively. Only significant correlations are shown. Negative corelations are in italics.

Table 3. Age-adjusted Spearman's correlations between the parameters investigated both on the begining and in termination of the trial.

	Steroids				Proteohormones			Lipid parameters							
	Cortisol	Testo	E-diol	A-dione	LH	FSH	SHBG	TC	HDL-C	LDL-C	AI	TG	Apo A-I	Apo B	Lp(a)
Cortisol				0.466											
Testo							0.716								
E-diol					−0.729										
A-dione	0.044														0.487
LH			0.000						0.616		−0.532		0.539		
FSH							0.651			0.573					
SHBG		0.001				0.003									
TC									−0.727	0.700	0.863	0.854	−0.500	0.803	
HDL-C					0.005			0.000		−0.743	−0.940	−0.734	0.767	−0.640	
LDL-C						0.013		0.001	0.000		0.811		−0.749	0.689	
AI					0.019			0.000	0.000	0.000		0.794	−0.661	0.762	
TG								0.000	0.000		0.000			0.691	
Apo A-I					0.026			0.041	0.000	0.001	0.004	0.000		−0.497	
Apo B								0.000	0.006	0.003	0.000	0.002	0.050		
Lp(a)				0.034											

The numbers above and below the diagonal represent correlation coefficients and their significance, respectively. Only significant correlations are shown. Negative correlations are in italics.

Discussion

Peroral supplementation of DHEA(S) is used to compensate for the decline of DHEA production with age. The effect of DHEA treatment on serum levels of androgens is reported to be gender-dependent. Morales *et al.* (1994) observed a twofold increase in serum levels of androgens (androstenedione, testosterone and dihydrotestosterone) after nocturnal oral 50 mg DHEA administration for 6 months in women and only a small rise in androstenedione in men. There was no change in circulating levels of sex hormone-binding globulin, estrone, or estradiol in either gender. The same authors reported later (Morales *et al.* 1998) that following the treatment of 100 mg DHEA for 6 months the DHEAS:cortisol ratio returned to pubertal values (10:1), in women, but not in men. While treated with DHEA, serum SHBG levels declined more in women (–40 %) than in men (–5 %). A decline of SHBG was also reported during transdermal DHEA treatment in women (Diamond *et al.* 1996).

In the present study, using 25 mg 7-oxo-DHEA *via* the transdermal route, we observed a significant decline in both main sex hormones together with a concomitant increase of LH. Other changes in hormonal patterns did not attain statistical significance. High-dose DHEA regimens usually lead to its transformation into active androgens with possible deleterious metabolic effects. Consequently, the current trend is to use low doses and we can speculate that our low parenteral challenge with 7-oxo-derivative rather suppresses its transformation to androgen products while maintaining its favorable metabolic properties.

Several authors have found that men with low levels of DHEA and DHEAS may be liable to development of fatal cardiovascular complications (Johannes *et al.* 1999, Poršová-Dutoit *et al.* 2000). The association with cardiovascular disease in women has been found either to be positive or absent. It is well known that such disorders are associated with altered levels of some lipids and lipoproteins (Poršová-Dutoit *et al.* 2000). The relationship between DHEA(S) and lipids was documented by epidemiological and cross-sectional studies as well as by investigation of the effects of DHEA(S) administration in humans (Poršová-Dutoit *et al.* 2000). Nestler *et al.* (1988) found significantly depressed serum total cholesterol levels in men treated with relatively large doses of DHEA (1600 mg daily) given for 28 days in a randomized, double-blind study

(mean values 4.82 *vs.* 4.48 mmol/l, respectively) which was almost entirely due to a fall of 7.5 % in mean serum LDL-C levels. Surprisingly, except for serum DHEA and androstendione, other sex hormones and SHBG did not change. Eleven years later, the same group of authors (Barnhart *et al.* 1999) supplemented postmenopausal women with 50 mg DHEA for 3 months and observed a non-significant decline in serum HDL-C and Lp(a) values. Replacement doses 100 mg of DHEA given for 3 months (Flynn *et al.* 1999) to older men led, concomitantly with the endocrine changes, to some small but significant variations in blood values including a decrease of total cholesterol and an increase in HDL-C. In cross-sectional studies (e.g. Okamoto 1998), DHEAS levels positively correlated with HDL-C and negatively correlated with LDL-C even after adjustment for age in both sexes. Furthermore, the mean atherogenic index significantly and inversely correlated with the rise of tertiles of the DHEAS level, both before and after adjustment for age, TC, HDL-C and TG.

In the study mentioned above, Morales *et al.* (1994) observed various, slight and insignificant changes in the lipid spectrum after peroral treatment with smaller dose of DHEA for a longer period. Recently, we applied DHEA in the form of a gel transdermally for 5 consecutive days to men and we measured the blood levels of steroids, gonadotropins, lipids and lipoproteins before the start of treatment, immediately after the last application and 5 weeks later (Šulcová *et al.* 2000). We found that DHEA significantly increased steroid sex hormones and significantly lowered LH levels. These effects might be adverse in older men especially in the relation to the risk of cancer. Moreover, we found almost no alterations in lipid and lipoprotein parameters.

In the present study, not only favorable (raised HDL-C, Apo A-I, decrease of TG and AI), but also negative changes [elevation in Lp(a) and Apo B] are documented. However, the difference in the last two parameters posses a relatively low power of significance, so that the protective effects, mainly an apparent decrease of atherogenic index, seem to be dominant. Lipoprotein(a) is considered to be a very stable, genetically determined lipid fraction and its major individual impairment is not probable. Table 3 shows a lot of significant relationships, especially at the end of the test period. The mutual, mostly expectable connections among the lipid parameters show a consistent, non-random shift through the entire lipid spectrum.

Correlation coefficients in the case of LH indirectly reflect a decline in sex steroid production.

Dehydroepiandrosterone exerts several physiological effects without involving its distal metabolites, namely steroid sex hormones (Lardy *et al.* 1995, Hampl *et al.* 1997, 2000). This led to the postulate that DHEA might include the precursor of steroids acting as mediators in these effects (Lardy *et al.* 1995, Hampl *et al.* 1997, 2000). Such derivatives of DHEA might be its 7-oxygenated metabolites, 7α-hydroxy-DHEA, 7β-hydroxy-DHEA and 7-oxo-DHEA (Lardy *et al.* 1995, Hampl *et al.* 1997, 2000). Lardy *et al.* (1995) demonstrated, that 7-oxygenated derivatives actively induce thermogenic enzymes in the rat liver and that the 7-oxo derivative is more potent than its parent steroids. These findings confirm the possibility of parallel non-genomic effect of DHEA and hence one could speculate about the reason of the surprisingly rapid influence (only one-week transdermal application) of 7-oxo-DHEA on serum lipid fractions.

Moreover, Waxman (1996) showed a dramatic increase in both size and number of liver peroxisomes in rodents given pharmacological doses of DHEA. This response is associated with a substantial induction of peroxisomal fatty acid β-oxidation and an activation of microsomal cytochrome P450 4A fatty acid hydroxylases (ω-oxidation). DHEA, its sulfate and 17β-reduced metabolite bind to PPARs (preferably PPARα), similarly as structurally diverse chemicals including many fatty acids, hypolipidemic drugs, insulin sensitizers and other xenobiotic compounds. These ligand-activated transcription factors belong to the steroid/thyroid/retinoid receptor superfamily. The affinity of the 7-hydroxy or 7-oxo DHEA to PPAR ligand is not known, but it seems to be higher than for original molecule (Lardy *et al.* 1995). Many impacts of the PPARs activation important for the considerations mentioned above have been described: a) induction of mitochondrial uncoupling proteins expression, b) control of a variety genes in several pathways of lipid metabolism, c) expression of the enterocytic fatty acid binding protein, d) an increase of alipoprotein lipase activity, e) up-regulation of Apo A-I and Apo A-II, major HDL lipoproteins, f) down-regulation of Apo C-III, an atherogenic components of Apo B containing lipoproteins, g) increase of liver and heart L-carnitine levels and carnitine acetyl transferase activity, and h) suppression of the NF-κB activity that is a key regulating transcription factor for main proinflammatory cytokines (Desvergne and Wahli 1999).

The aim of this study was to investigate the effect of transdermally applied 7-oxo-DHEA on hormones and especially on lipid and lipoprotein levels in men. We administered 25 mg 7-oxo-DHEA daily for 5 days to healthy men of various ages and we found significantly lower levels of testosterone and estradiol and significantly increased LH levels. Contrary to our recent finding concerning the transdermal DHEA application, the profound effect of 7-oxo derivative given by the same route was rather surprising. Total cholesterol and atherogenic index decreased, whereas HDL-C and Apo A-I went up. All these changes were significant, in the case of total cholesterol the decline was close to significance. Triglycerides and LDL-C values also fell, but not significantly. The changes seen in our trial, particularly those concerning the lipid components, can act protectively on the arterial wall and consequently on the cardiovascular system. Moreover, the ointment (gel) application containing 7-oxo-DHEA might be a suitable form of drug supplementation in elderly men.

Abbreviations

A-dione	androstenedione
AI	atherogenic index
Apo A-I	apolipoprotein A-I
Apo A-II	apolipoprotein A-II
Apo C-III	apolipoprotein C-III
Apo B	apolipoprotein B
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
E-diol	estradiol
FSH	follicles-stimulating hormone
HDL-C	high density lipoprotein cholesterol
IRMA	immunoradiometric assay
LDL-C	low density lipoprotein cholesterol
LH	luteinizing hormone
Lp(a)	lipoprotein(a)
NF-κB B	nuclear factor-κ
PPAR	peroxisome proliferator-activated receptor
SHBG	sex hormone-binding globulin
TC	total cholesterol
Testo	testosterone
TG	triglycerides (triacylgliceroles)

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