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Effects of Transdermal Application of DHEA on the Levels of Steroids, Gonadotropins and Lipids in Men

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Summary

In order to ascertain the kinetics of absorption and metabolism of transdermally administered dehydroepiandrosterone (DHEA), 10 men 29-72 years old (mean 52.4±14.5) received 50 mg DHEA/day in a gel applied onto the skin of the abdomen for 5 consecutive days. The objective was to establish the extent to which DHEA influences the levels of gonadotropins, sex hormone-binding globulin and lipids. It was found that DHEA is well absorbed and rapidly metabolized to its sulfate (DHEAS), androstenedione, and consequently to testosterone and estradiol. The DHEA levels that markedly increased after the first doses gradually declined already during the application, and this decline proceeded even after it was discontinued, reaching levels significantly lower than the original ones. On the other hand, the levels of DHEA metabolites (with the exception of DHEAS) rose during the application and reached values significantly higher than the basal ones within 5 weeks. This effect was accompanied by significantly decreased levels of LH. The serum levels of lipids, namely of cholesterol (both HDL and LDL cholesterol), triglycerides, apolipoproteins A-I and B and lipoprotein(a) after DHEA application were not changed significantly, and the atherogenic index (AI) remained unaltered. However, some correlations between hormones and lipids were found. Negative correlations concerned the following indices: DHEA/Lp(a); DHEAS/cholesterol; DHEA, DHEAS, testosterone/TG; testosterone/AI. On the other hand, LH, FSH/cholesterol, FSH, SHBG/LDL cholesterol, FSH/Apo B, Lp(a) correlated positively. It can be concluded that transdermal short-time application of DHEA results in a decrease of endogenous DHEA after finishing the treatment, with a parallel marked increase in the levels of sex hormones. Using this application protocol, exogenous DHEA neither altered the lipid spectrum, nor did it influence the atherogenic index.

Key words
DHEA • Transdermal • Steroids • Gonadotropins • Lipids • SHBG

Introduction

Although dehydroepiandrosterone (DHEA) and especially its sulfate belong among the most abundant circulating steroids, their physiological role has not yet been fully elucidated. Since Baulieu (1996) introduced the term "fountain of youth" for DHEA, an interest in this

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steroid has increased not only among experts, but also among non-professionals. It is well known that many people, especially men, take DHEA regularly for improving their well-being and good physical condition. In spite of this fact, there is scanty knowledge on the changes in biochemical parameters, especially in hormonal and lipid spectra, caused by DHEA intake. The findings from individual studies are often controversial, among others because clinical trials, doses and forms of DHEA application differ considerably. Thus men received 50, 100, 200 or 1600 mg DHEA, respectively, in a single dose or daily, for a period ranging from two weeks to 3 months, mostly perorally (Nestler *et al.* 1988, Morales *et al.* 1994, Young *et al.* 1997, Wolf *et al.* 1997, Arlt *et al.* 1999, Flynn *et al.* 1999). Labrie *et al.* (1996) compared the androgenic effects of DHEA administered perorally, percutaneously and by hypodermic injection in an experiment on rats. They concluded that peroral application caused only 3 % effect of that achieved by subcutaneous application, while percutaneous administration resulted in a 33 % increase, i.e. in an 11-fold higher effect. In women, transdermal replacement therapy with estrogens belongs to common practice and this approach has also been tried with DHEA supplementation (Diamond *et al.* 1996, Labrie *et al.* 1997). In men, transdermal application of DHEA has been used in one case only, in which the steroid was applied in the form of a 20 % solution (Labrie *et al.* 1997). DHEA in the form of an ointment or gel has not yet been administered.

The above authors investigated the steroid profiles and the levels of selected lipids, as well as the physical condition and well-being after DHEA application. The only findings reported by all these authors concerned increased blood levels of DHEA, DHEAS and androstenedione. Either a significant (Young *et al.* 1997, Wolf *et al.* 1997, Arlt *et al.* 1999) or no effect (Nestler *et al.* 1988, Morales *et al.* 1994, Labrie *et al.* 1997, Arlt *et al.* 1999) of DHEA application on the levels of testosterone and estradiol were reported. In addition, the observed changes of lipid spectrum differed considerably, but these parameters have been followed up in several studies only (Nestler *et al.* 1988, Morales *et al.* 1994). In the latter case only slight, insignificant changes were recorded (Morales at al. 1994).

We were interested whether short-term administration of DHEA, when applied by a modern transdermal route, would influence hormonal and lipid spectra. Therefore, dehydroepiandrosterone was applied in the form of gel for five consecutive days to 10 male volunteers of different age. The levels of some steroids, proteohormones and lipid parameters have been measured before, during and after DHEA application.

Subjects and Methods

Steroids and chemicals

Dehydroepiandrosterone (3β-hydroxy-5-androstene-17-one) was purchased from Sigma (St. Louis, MO, USA). Diethyl ether and chemicals used for radioimmunoassay, all analytical grade, were from Merck (Darmstadt, Germany).

Subjects

The group of probands was recruited from ten informed healthy male volunteers aged 29-72 years (52.4±14.5) (mean ± S.D.). None of them took any drugs and had no health problems except the higher age.

Treatment protocol

Dehydroepiandrosterone was applied in the form of a gel containing 1 g DHEA/100 g. Approximately 5 g of the gel, corresponding to 50 mg DHEA a day, was applied transdermally on the abdominal skin before sleeping at 21:00 h, for 5 consecutive days. Fasting blood collections were always performed in the morning, before treatment (Day 0), during the experiment after 3 days of application (Day 4), on the next day after application of the last dose (Day 6) and 5 weeks after termination of DHEA administration (Day 42). In four subjects (age 55-72, 61.8±8.3 years) the removal data during the experiment (Day 4) and after 5 weeks (Day 42) were not obtained for technical reasons. The age of males who completed the experiment was 29-69 years (46.2±14.8 years).

Steroid determination

DHEA, its sulfate and estradiol were determined in the sera by commercial RIA kits from Immunotech (Praha, Czech Republic). Other steroids (cortisol, testosterone, androstenedione) were determined by RIA using antisera prepared in the authors' laboratory and either commercial or in the laboratory radioiodinated radioligands (Putz *et al.* 1982, Bičíková *et al.* 1988, Hill *et al.* 1996).

Determination of proteohormones

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

Determination of lipids

Serum total cholesterol, triglycerides and HDL cholesterol levels were measured enzymatically using kits purchased by Roche Boehringer Mannheim. LDL cholesterol was calculated by Friedewald's formula, serum apolipoprotein A-I, apolipoprotein B and lipoprotein(a) assessment was performed according to the Laurent's rocket method using Behring antisera.

Statistical analysis

For the evaluation of differences in serum levels of the parameters under study between the individual stages of the experiment, two-factor ANOVA was used. The first and the second factor expressed the differences between stages of the experiment and the individual differences, respectively. The robust Wilcoxon paired test was used for comparison of the paired differences in the studied substances between days 0 and 6.

Most of the variables were transformed by logarithmic or power transformation prior to the statistical tests to stabilize the group variances and to approximate the data distributions to Gaussian, which is the prerequisite for ANOVA testing. The mutual relations between the parameters studied were evaluated using robust Spearman's correlations.

Table 1. The levels of steroids, gonadotropins (LH, FSH) and sex hormone-binding globulin (SHBG) before and after 5 days of transdermal treatment by DHEA in men of various age.

Substance	Before application	After application	Significance of the differences
DHEA (nmol/l)	13.04±2.05	19.93±2.08	p<0.003
DHEAS (μmol/l)	4.55±0.56	5.82±0.72	p<0.006
*Androstenedione (nmol/l)**	2.94±0.42	5.33±1.01	p<0.06 (NS)
Testosterone (nmol/l)	13.48±0.99	14.41±1.37	NS
Estradiol (nmol/l)	0.109±0.026	0.187±0.063	NS
Cortisol (nmol/l)	626.8±47.9	573.5±63.1	NS
LH (IU/l)	2.39±0.31	4.07±1.21	NS
FSH (IU/l)	3.49±0.93	3.35±0.98	NS
SHBG (nmol/l)	23.8±2.95	22.06±2.44	NS

*Data are mean ± S.E.M. Number of subjects is 10 (except of * n = 7). The robust Wilcoxon paired test was used for comparison of the paired differences between stages in which the parameters were followed.*

Results

Hormonal profiles

Differences between basal levels and those measured after termination of the treatment are shown in Table 1. The changes in the levels of selected steroids and proteohormones in all 10 men listed according to their age after five days of transdermal application of DHEA are apparent from Figure 1. Ten hours after the last application, the only significantly elevated levels were those of DHEA and its close metabolite, DHEA sulfate (DHEAS). The increase of another immediate metabolite, androstenedione, was only on the fringe of statistical significance. A tendency to increased values was observed in sexual hormones, testosterone and estradiol, but these changes were not significant, likewise the decrease of cortisol levels. An insignificant increase of the mean LH levels was observed. FSH levels were not changed (with the exception of three outliers). The mean SHBG levels decreased, but also non-significantly. A well-known decline of DHEA and DHEAS with increasing age (Šulcová *et al.* 1997) was recorded even in this small group. In all the hormonal parameters studied, except SHBG, individual differences were observed especially in both sex steroids in response to DHEA treatment, irrespectively of the age.

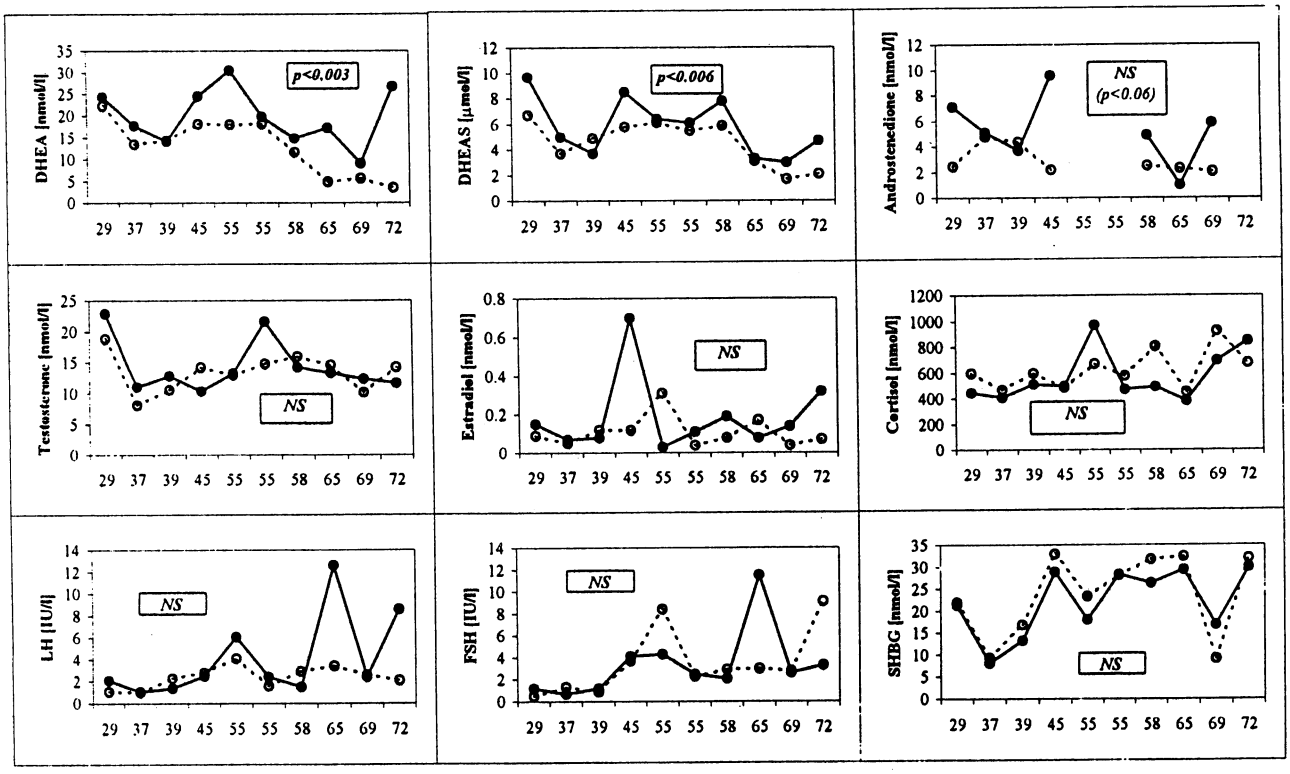

Fig. 1. *Individual levels of selected steroids, gonadotropins and SHBG in ten male volunteers before and immediately after five days of transdermal application of DHEA. Open circles, dotted lines = Day 0 (before application); full circles, full lines = Day 6 (10 h after the last application). The age of subjects is indicated on the abscissa. The results of statistical evaluation of the differences between Days 0 and 6, as determined by Wilcoxon paired test, are in the frames.*

The time course of hormonal levels during and after DHEA administration is shown in Figure 2 which illustrates the widely different response of the studied hormones to steroid administration. The levels of DHEA itself increased substantially at the beginning of the treatment, but they already started to decline after the third dose, so that the levels no longer differed significantly from the basal ones 10 h after terminating the application. This decline progressed and the mean levels of DHEA reached values significantly lower than the basal one 5 weeks after the application. Direct DHEA metabolites, namely its sulfate and androstenedione, did not follow this trend. DHEAS levels rose significantly after the first few doses and then moderately and insignificantly declined, reaching approximately the basal levels 5 weeks later. On the other hand, the concentration of androstenedione was significantly enhanced at the beginning and this tendency (though no longer significant) continued even after the termination of DHEA administration reaching more than twofold levels after 5 weeks. The sexual hormones exhibited a similar time course like androstenedione. Testosterone levels increased moderately during DHEA application, but this rise proceeded and after 5 weeks they differed significantly from the basal values (an increase by almost 50 %). The rise of estradiol levels was more rapid at the beginning and it continued after DHEA treatment had been discontinued. Its final concentration was about threefold higher than the initial values, and, in spite of great individual variations, the differences were significant (Figs 1 and 2). The changes of LH levels corresponded to those of sexual hormones. After a moderate initial increase following the first doses of DHEA they declined significantly amounting only about a half of the original value. The FSH levels also slightly rose at first, but they already returned to the basal levels during the treatment and did not change later. The cortisol levels displayed a tendency to decrease, but this decline was insignificant and they returned to basal values after termination of the application. The levels of SHBG declined very slightly during the treatment and they were unchanged after the experiment had been discontinued.

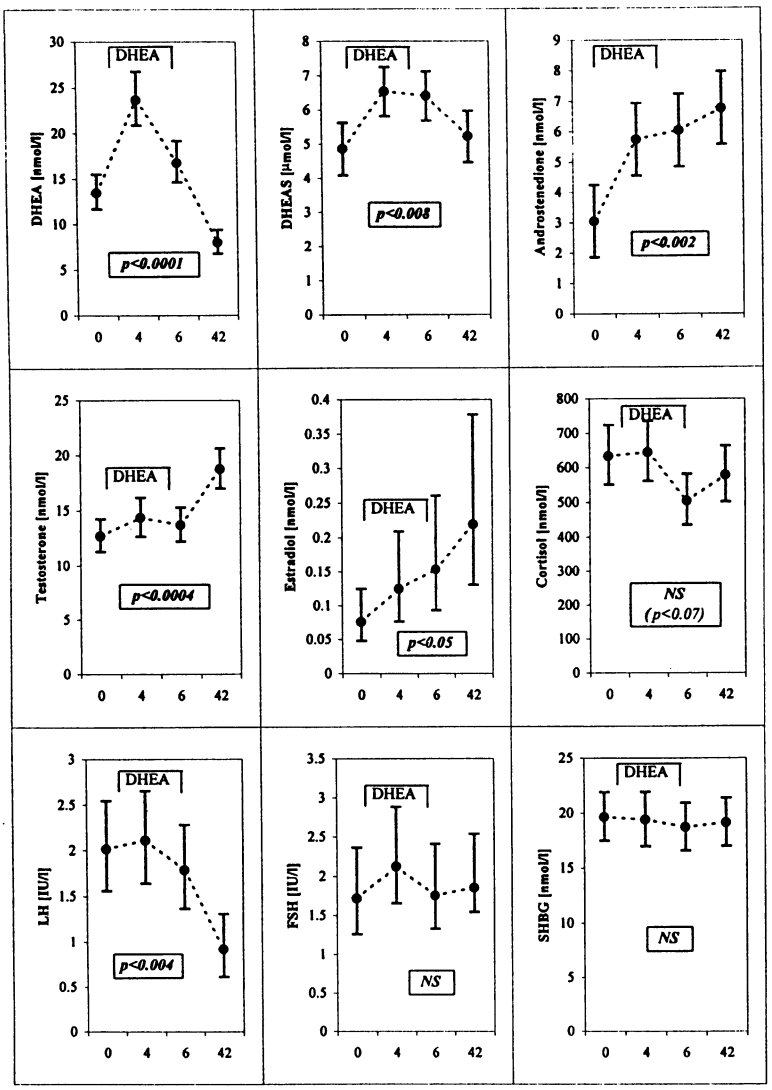

Fig. 2. *The time course of average hormonal levels in male volunteers before, during and after transdermal application of DHEA. The abscissa shows the days of the experiment: 0 = before application, 4 = after 3 days of treatment, 6 = after 5 days of treatment, 42 = 5 weeks after the last application of DHEA. The number of subjects was six. Full circles express the means, full straight lines the 95% confidence intervals of transformed mean values. An overlap of these intervals indicates non-significant difference between the periods. Two-factor ANOVA after transformation of the variables to minimum skewness was employed for the evaluation of the data. Statistical significance of the first factor, taking into consideration only the stages of the experiment, is in the frames. (The second factor only explains the inter-individual differences).*

Lipids

The time course of the levels of cholesterol, HDL and LDL cholesterol, triglycerides and lipoproteins (Apo A-I, Apo B, Lp(a) as well as the values of atherogenic index before, immediately after, and 5 weeks following the termination of DHEA application, respectively, are shown in Figure 3. It is obvious that the changes in the lipid spectrum after 5 days of DHEA application were only moderate and insignificant. However, total, HDL and LDL cholesterol displayed a tendency to decline and after the end of the treatment their levels did not completely return to initial values even after 5 weeks. The atherogenic index that was calculated as the cholesterol/HDL ratio remained unchanged during the whole period. The changes of triglyceride levels varied in each individual. Only a slight decrease of the average value was observed immediately after application of DHEA, but this value later returned to the initial level. Apolipoproteins A-I a B did not respond to DHEA treatment. The level of lipoprotein(a) rose non-significantly during the treatment and did not change for the following 5 weeks.

Correlation between hormones and lipids

The mutual correlations in all paired combinations of the parameters studied (summarized for all levels of the experiment, i.e. irrespectively of the time of sample collection) are shown in Table 2. The coefficients of correlation, the number of pairs taken into the calculation and the levels of statistical significance are given in Table 2. Only the significant correlations are shown.

Steroids. The following pairs of variables correlated positively: DHEA with DHEAS and with androstenedione; DHEAS with androstenedione and with testosterone; androstenedione with estradiol. Testosterone

also correlated positively with SHBG. As far as the correlation of steroids with lipids is concerned, only negative correlations were found: DHEA, DHEAS and testosterone with triglycerides; DHEA with lipoprotein (a); DHEAS with total cholesterol; testosterone with the atherogenic index.

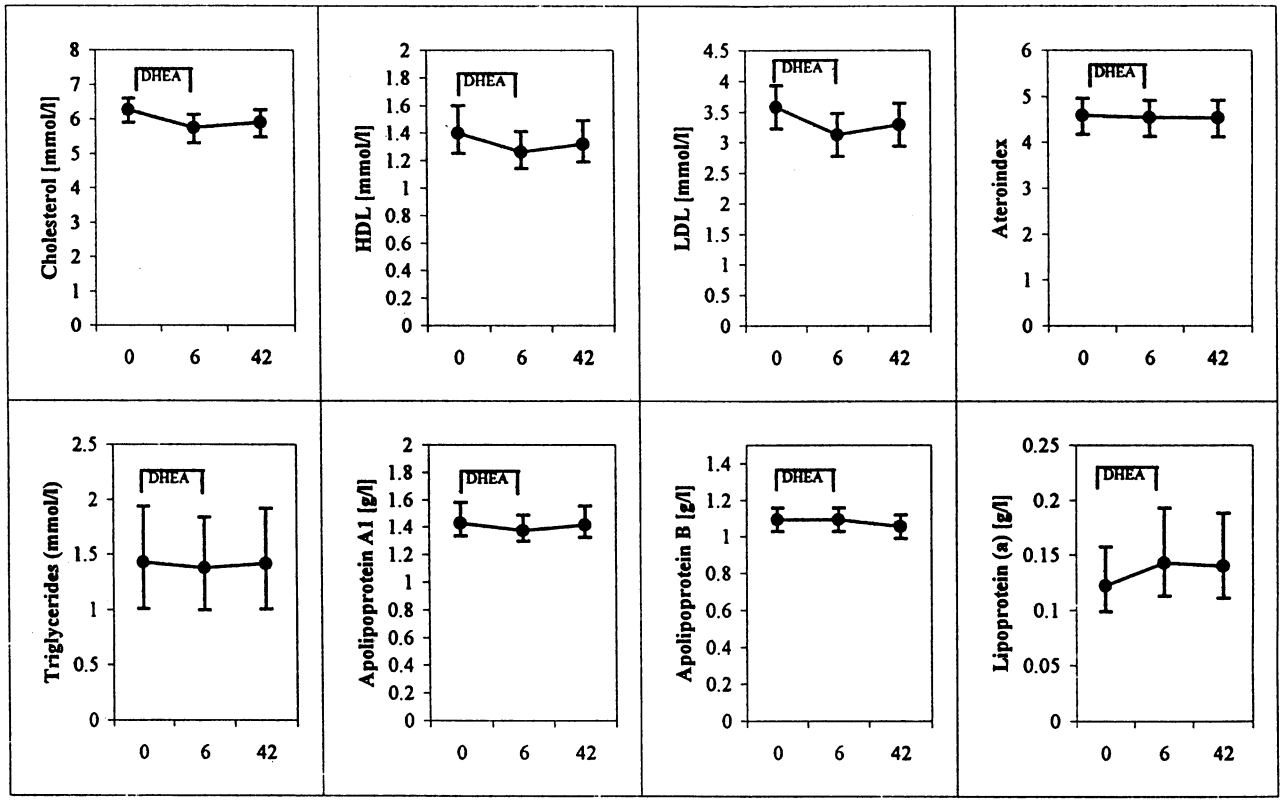

Fig. 3. *The time course of average lipid parameters in male volunteers before and after transdermal application of DHEA. The abscissa shows the days of the experiment: 0 = before application, 6 = immediately after 5 days of treatment, 42 = five weeks after the last application of DHEA (six subjects in the group). Full circles express the means, full straight lines the 95% confidence intervals of retransformed mean values. An overlap of these intervals indicates non-significant difference between two stages of the treatment. Two-factor ANOVA after transformation of the variables to minimum skewness was employed for the evaluation of the data. Statistical significance of the first factor, taking into consideration only the stages of the experiment, is in the frames. The second factor only explains the inter-individual differences. No significant differences have been found between the stages of the experiment in any of the compounds studied.*

Gonadotropins and SHBG. LH, FSH and SHBG correlated positively with each other. Several positive correlations were found between proteohormones and lipids. In all instances, these correlations dealt with "undesirable" lipids, namely total cholesterol, LDL cholesterol, apolipoprotein B and lipoprotein(a). FSH correlated positively with all the latter lipids, while other proteins with only some of them.

Lipids. Correlations between some lipids were very strong and mostly positive. Cholesterol correlated with triglycerides and with Apo B, HDL cholesterol with Apo A-I, LDL cholesterol with Apo B and with AI, atherogenic index with Apo B. As expected, the only negative correlations were found between AI and HDL cholesterol and between AI and Apo A-I.

When the data obtained after administration of DHEA only were evaluated (n=6; the results are not presented graphically), some of the negative correlations were strengthened, namely: DHEAS/TG ($r = -0.943$, $p<0.035$) and testosterone/AI ($r = -1.000$, $p<0.0001$).

Table 2: Correlations between individual steroids, proteohormones and lipid parameters totally in the group of men with the application of DHEA

	STEROIDS						PROTEO -HORMONES			LIPID PARAMETERS							
STEROIDS	**DHEA**	**0.765** *n = 26*	**0.527** *n = 23*											**-0.586** *n = 13*			**-0.62** *n = 13*
	n = 26 **0.0001**	**DHEAS**	**0.547** *n = 23*	**0.445** *n = 26*						**-0.615** *n = 13*				-0.791 *n = 13*			
	n = 23 **0.0135**	*n = 23* **0.0103**	**A-dion**		**0.569** *n = 23*												
		n = 26 **0.026**		**Testo**					**0.565** *n = 25*				**-0.63** *n = 13*	**-0.768** *n = 13*			
			n = 23 **0.0076**		**E-diol**												
						Cortisol											
PROTEO-HORMONES							**LH**	**0.726** *n = 26*	**0.474** *n = 25*	**0.571** *n = 13*							
							n = 26 **0.001**	**FSH**	**0.632** *n = 25*	**0.764** *n = 13*		**0.797** *n = 13*				**0.674** *n = 13*	**0.613** *n = 13*
				n = 25 **0.0056**			*n = 25* **0.0203**	*n = 25* **0.002**	**SHBG**			**0.725** *n = 13*					
LIPID PARAMETERS		*n = 13* **0.033**					*n = 13* **0.0479**	*n = 13* **0.0082**		**Chol.**				**0.604** n = 13		**0.754** *n = 13*	
											HDL chol.		**-0.806** *n = 13*		**0.945** *n = 13*		
								n = 13 **0.0058**	*n = 13* **0.0156**	*n = 13* **0.012**		**LDL chol.**	**0.577** *n = 13*			**0.922** *n = 13*	
				n = 13 **0.0291**							*n = 13* **0.0052**	*n = 13* **0.0457**	**AI**		**-0.79** *n = 13*	**0.663** *n = 13*	
	n = 13 **0.0424**	*n = 13* **0.0061**		*n = 13* **0.0078**						*n = 13* **0.0363**				**TG**			
											n = 13 **0.0011**		*n = 13* **0.0062**		**Apo A1**		
								n = 13 **0.0196**		*n = 13* **0.009**		*n = 13* **0.0014**	*n = 13* **0.0216**			**Apo B**	
	n = 13 **0.0318**							*n = 13* **0.0336**									**Lp (a)**

The bold values above and below the diagonal represent the significant Spearman's correlation coefficients and the the levels of statistical significance, respectively.

Discussion

One of the conclusions of our recent review (Poršová-Dutoit *et al.* 2000) on the protective role of DHEA/DHEAS in the pathogenesis of atherosclerosis and coronary heart disease concerned the finding that men with low levels of DHEA and DHEAS may be prone to the development of fatal cardiovascular events. Because such disorders are always associated with altered levels of certain lipids and lipoproteins, a number of authors have looked for a relationship between DHEA(S) and lipid metabolism. The results were controversial: positive, negative as well as no correlations have been found (Poršová-Dutoit *et al.* 2000). A question of the usefulness and efficacy of DHEA supplementation in aged people also remains unresolved. Nestler *et al.* (1988) found significantly lowered levels of total cholesterol and LDL cholesterol after 4 weeks of a peroral massive dose of DHEA (1600 mg/day) to men. Morales *et al.* (1994) observed various non-significant changes in the lipid spectrum after a longer treatment period of a smaller dose (50 mg/day for 12 weeks). In other studies where DHEA was given to men, lipids and lipoproteins were not followed.

The aim of this study was to find out how dehydroepiandrosterone is absorbed and metabolized after transdermal administration, and whether it influences the lipid spectrum. The level of DHEA in the blood serum of subjects was increased on the average by 53 % after 5 days of treatment, but even a higher level (77 % increase) was attained as early as after 3 doses. It means that percutaneously applied DHEA enters rapidly into the circulation and it is quickly metabolized. Indeed, its short metabolic half-time is known (Baulieu 1996, Khorram 1996). The direct metabolites of DHEA are its sulfate (DHEAS), and the main product of the action of enzyme 3β-hydroxysteroid dehydrogenase, androstenedione. The levels of both these metabolites rose considerably following the first three DHEA doses, but their further time course was different. DHEAS followed to some extent DHEA levels, which declined rapidly since day 4, while the concentration of androstenedione further rose and this rise was followed by concentrations of its metabolites, testosterone and estradiol. It is of interest that the decline of DHEA as well as the rise of its physiologically effective metabolites (sexual hormones) continued even after termination of the treatment for a relatively long period (5 weeks). It seems as if increased DHEA levels would initiate processes in the organism, leading to the production of sexual hormones. It resembles the situation in adrenarche and puberty. In this mechanism of the "late onset of pseudopuberty" no increase of LH occurred, but, on the contrary, increased levels of sexual hormones caused its decrease. Thus no "rebound phenomenon", but another mechanism is involved here. The decline of serum LH following DHEA administration has been described by Labrie *et al.* (1996) in an experiment on ovariectomized rats.

Morales *et al.* (1994) observed a significant decrease of SHBG, but the levels of testosterone after 12 weeks of DHEA administration to men were not altered. In our experiment, we have found only a mild and insignificant decline of SHBG after 5 days of DHEA application. A considerable increase of testosterone occurred even after a certain delay since the termination of DHEA application. Wolf *et al.* (1997) found a significant increase in serum testosterone after 14 days of peroral administration of 50 mg DHEA to both males and females. Furthermore, Young *et al.* (1997) obtained similar results after a single dose of 200 mg DHEA given to patients with panhypopituitarism. The increase of testosterone levels following DHEA administration is an important finding, especially with respect to the high negative correlation between testosterone and atherogenic index. On the other hand, the tendency of SHBG to a decline could be important in connection with the positive correlation between SHBG and LDL cholesterol. Increasingly more authors accept the hypothesis that various effects of DHEA(S), which are not directly connected with their role as precursors of sexual steroid hormones, are mediated by their metabolites oxygenated at carbon C7 (Hampl *et al.* 1997, 2000). Transformation of DHEA to its 7-hydroxylated derivatives during and after transdermal application of DHEA to men, as well as the possible effects of these metabolites, was the subject of another paper from our group (Hampl *et al.* 2000).

Acknowledgements

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Abbreviations

AI	atherogenic index
Apo	apolipoprotein
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
FSH	folicules stimulating hormone
HDL	high density lipoprotein
LDL	low density lipoprotein
LH	luteinizing hormone
Lp(a)	lipoprotein(a)
SHBG	sex hormone-binding globulin
TG	triglycerides

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Reprint requests

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