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How Short-term Transdermal Treatment of Men with 7-oxo-dehydroepiandrosterone Influences Thyroid Function

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Summary

Dehydroepiandrosterone may influence thyroid function. Its metabolite, 7-oxo-dehydroepiandrosterone, a precursor of immunomodulatory 7-hydroxylated metabolites and thermogenic agent, belongs to candidates of steroid replacement therapy. The question was addressed whether its application does influence laboratory parameters of thyroid function. 7-Oxo-dehydroepiandrosterone in the form of emulgel, 25 mg/day, was applied transdermally to 21 healthy men for 8 consecutive days. Morning blood was collected before the treatment (Day 0, Stage 1), during treatment (Day 5, Stage 2), on the first day after the last administration (Day 9, Stage 3), one week (Day 16, Stage 4), and 9 weeks (Day 72, Stage 5) after treatment termination. The levels of thyrotropin, free thyroxine and triiodothyronine, dehydroepiandrosterone, its sulfate and its 7-hydroxyepimers were measured. The changes were evaluated by analysis of variance and correlation analysis. During treatment a significant rise of 7β-hydroxy-dehydroepiandrosterone was observed, which persisted 1 week after treatment termination. No changes were observed in dehydroepiandrosterone and its sulfate. Though a slight but significant rise of TSH and of both thyroid hormones occurred during treatment, its levels soon returned to the basal values. It was concluded that treatment of 7-oxo- dehydroepiandrosterone affects the thyroid parameters only temporarily and that it provides a considerable persistent amount of 7β-hydroxy-dehydroepiandrosterone.

Key words

7-oxo-dehydroepiandrosterone • Transdermal application • Thyroid hormones

Introduction

Plasma levels of dehydroepiandrosterone (DHEA), its precursors and metabolites may be altered in thyroid disorders. Tagawa *et al.* (2000, 2001) demonstrated that in hypothyroid patients the levels of DHEA, its sulfate (DHEAS), pregnenolone sulfate, and both unconjugated and sulfated androstenediols were significantly lower than in age- and sex-matched healthy controls, while only the sulfated steroids were increased in hyperthyroidism. Altered DHEA levels were recently reported in men with hyperlipidemia, known as a risk factor in hypothyroidism (Šulcová *et al*. 2005).

It is also known that DHEA and some of its metabolites act as immunomodulators, in some instances counteracting the exaggerated effects of glucocorticoids on the immune system (Kalimi *et al.*1994, Kalimi and Regelson 2000). Recent reports from our and other

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laboratories have demonstrated that 7-oxygenated DHEA metabolites may be even more effective locally active immunoprotective agents than DHEA itself (for review see Morfin 2002).

In our recent paper (Hampl *et al.* 2003) we have pointed to a negative correlation between one of 7-hydroxylated DHEA metabolites, 7β-hydroxy-dehydroepiandrosterone (7β-OH-DHEA) and free triiodothyronine (fT_3) in euthyroid healthy subjects.

Both 7-hydroxylated DHEA metabolites are present in human blood in low nanomolar concentrations (Hampl *et al.* 2001) together with an intermediate of their interconversion, 7-oxo-dehydroepiandrosterone (7-oxo-DHEA) (Marwah *et al.* 1999, Robinzon *et al.* 2001). When administerd to humans, 7-oxo-DHEA is metabolized to both 7-hydroxyepimers of DHEA, and as such may be one of several candidates of steroid replacement therapy (Hampl *et al.* 2000). In some countries (e.g. in the USA) it is available without prescription under the trademark 7-keto DHEA. Moreover, experimental evidence has been reported that this steroid is a potent thermogenic agent and it could reverse the effect of thyroid hormone removal on thermogenesis (Bobyleva *et al.* 1997). It was even suggested for prevention of Raynaud´s attacks (abnormal digital vasoconstriction in response to cold) (Ihler and Chami-Stemman 2003).

With respect to the potential beneficial effects of 7-oxo-DHEA, we have been studying its various endocrine effects when given transdermally to male volunteers in the form of a well tolerated emulgel. One of the questions was whether, and if so how, does 7-oxo-DHEA administration influence the thyroid laboratory parameters.

Methods

Subjects

The group of volunteers consisted of 21 informed male volunteers aged 20-70 years (44.8±14.4, mean ± S.D.). They were neither on regular medication nor had a health risk except for the higher age of some of them. The subjects were divided into small groups of 2-4 men each, who underwent treatment and blood collection together. The interval between treatments of successive groups was two weeks. The purpose of this arrangement was to avoid the seasonal effects as well as possible actual fluctuation of analytical methods. The whole study thus lasted 10 months.

Treatment protocol

7-oxo-DHEA was applied transdermally as an emulgel containing 0.5 g of 7-oxo-DHEA per 100 g (purchased from A. Nováček, Bochemie Inc., Bohumín, Czech Republic). The gel (5 g, corresponding to a daily dose of 25 mg 7-oxo-DHEA) was applied onto an abdominal skin area before sleeping at 22:00 h, for 8 consecutive days. Morning blood was collected after a night fast as follows: before the start of the treatment (Day 0, Stage 1), in the course of treatment (Day 5, Stage 2), on the first day after the last application (Day 9, Stage 3), one week- (Day 16, Stage 4), and 9 weeks (Day 72, Stage 5) after termination of the treatment, respectively. Blood sera were frozen and stored at –20 °C till the analysis.

Hormone analyses

Serum TSH, fT_4, and fT_3 were measured by ECLIA from Roche Diagnostics GmbH, Mannheim, Germany, using commercial Elecsys System 2010. DHEA and its sulfate were determined by RIA, using the kits from Immunotech (Prague Division, Czech Republic). The coefficients of variation for all the assays corresponded to the values declared by manufacturers. 7α- and 7β-OH-DHEA were measured by RIAs developed in the author's laboratory (Lapčík *et al.* 1998, 1999).

Statistics

For evaluation of the differences between the stages of the experiment, repeated measures one-way ANOVA was used with stage as a within-factor. Special care was given to data pretreatment respecting non-Gaussian data distribution, heteroscedascity (non-constant variance) and non-homogeneity. The data were transformed by power transformation to attain minimum skewness of studentized residuals. To eliminate the influence of outliers, only the data with an absolute studentized value less than 2.5 were considered. The ANOVA was completed using statistical software Statgraphics Plus 5 (Manugistics, Rockville, MD, USA).

Results

Changes of steroid levels before (Day 0, Stage 1), during (Day 5, Stage 2) and after (Days 9, 16 and 72, Stages 3-5) application of 7-oxo-DHEA to 21 male volunteers are shown in Figure 1, the corresponding levels of thyroid parameters can be found in Figure 2.

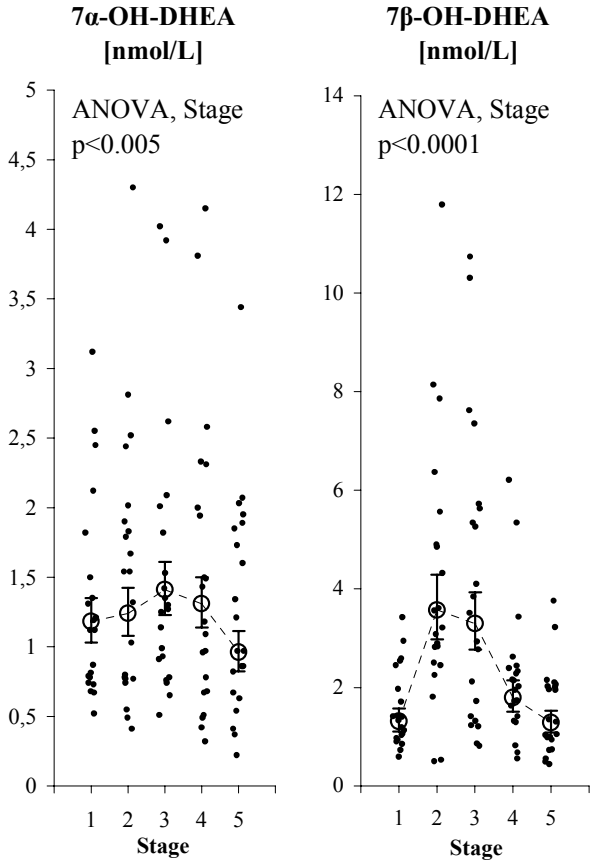

Fig. 1. Changes of serum levels of 7-OH-DHEA epimers before, during, and after transdermal application of 7-oxo-DHEA to 21 healthy males. Repeated measures ANOVA with stage of treatment as the within-factor was used for evaluation of the significance of the changes. Careful data analysis and the residual analysis were performed prior to test, to identify non-Gaussian data distribution and heteroscedascity. In the case of skewed data distribution and/or non-constant variance, the original data were transformed to minimum skewness before the analysis. The data with the absolute standardized residuals or absolute standardized values within individual stages greater than 3 were excluded from the computations. To avoid a masking effect of the most pronounced outliers, repeated outlier exclusion was used. Maximum number of excluded experimental points never exceeded 5 % of the total. The small full circles represent experimental points, the big empty circles with error bars represent transformed group means with 95 % confidence intervals after re-transformation to the original scale.

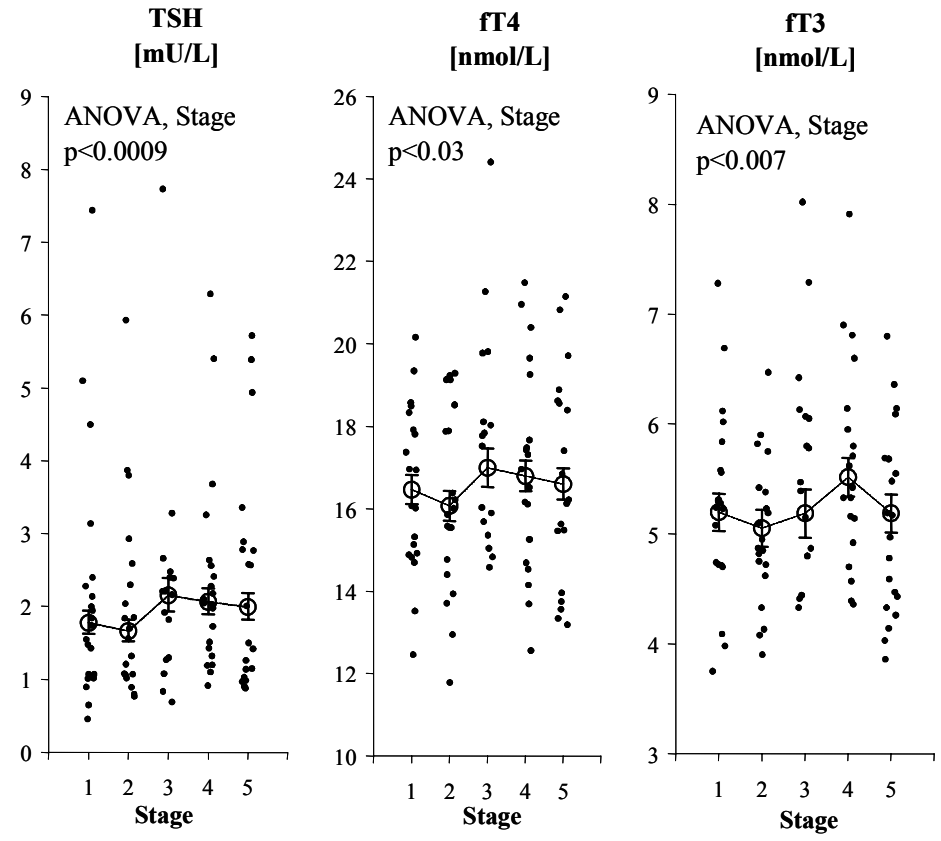

Fig. 2. Changes of serum levels of three major thyroid markers (TSH, fT3 and fT4) before, during, and after transdermal application of 7-oxo-DHEA to 21 healthy males. The statistical analysis was the same as described in the legend of Figure 1.

Table 1. Spearman's correlation matrix of the relations between DHEA, its 7-hydroxylated metabolites, thyroid markers, and age; basal values. The upper right parts from the diagonal represent simple pair- while the lower left parts the partial correlations. Each cell from above represents coefficient of correlation, significance and number of pairs. The significant correlations are shaded.

Age	-0.682 0.001 21	-0.588 0.005 21	-0.626 0.002 21	-0.633 0.002 21	-0.406 0.068 21	-0.280 0.219 21	-0.158 0.495 21
-0.140 0.618 21	**DHEA**	0.704 0.000 21	0.600 0.004 21	0.653 0.001 21	0.424 0.056 21	0.222 0.333 21	-0.014 0.953 21
-0.280 0.312 21	0.456 0.088 21	**DHEAS**	0.381 0.089 21	0.567 0.007 21	0.305 0.179 21	0.545 0.011 21	0.196 0.396 21
-0.384 0.158 21	0.198 0.480 21	-0.428 0.112 21	**DHEA7α**	0.712 0.000 21	0.260 0.255 21	0.207 0.367 21	0.072 0.755 21
0.087 0.759 21	0.069 0.807 21	0.406 0.133 21	0.703 0.003 21	**DHEA7β**	0.208 0.366 21	0.147 0.524 21	0.138 0.552 21
-0.235 0.398 21	0.099 0.726 21	0.211 0.450 21	0.129 0.646 21	-0.213 0.447 21	**TSH**	-0.152 0.511 21	-0.209 0.363 21
0.050 0.858 21	-0.245 0.379 21	0.651 0.009 21	0.375 0.168 21	-0.374 0.170 21	-0.304 0.270 21	**FT4**	0.409 0.065 21
-0.156 0.578 21	-0.087 0.759 21	-0.065 0.817 21	-0.132 0.639 21	0.201 0.472 21	-0.098 0.729 21	0.274 0.324 21	**FT3**

Table 2. Spearman's correlation matrix of the relations between DHEA, its 7-hydroxylated metabolites, thyroid markers, and age; all the data obtained during and after treatment. The upper right parts from the diagonal represent simple pair- while the lower left parts the partial correlations. Each cell from above represents coefficient of correlation, significance, and number of pairs. The significant correlations are shaded.

Age	-0.663 0.000 84	-0.593 0.000 84	-0.453 0.000 84	-0.139 0.206 84	-0.403 0.000 77	-0.254 0.027 76	-0.118 0.312 76
-0.277 0.014 84	**DHEA**	0.703 0.000 84	0.378 0.000 84	0.021 0.847 84	0.274 0.016 77	0.267 0.020 76	0.197 0.088 76
-0.273 0.016 84	0.522 0.000 84	**DHEAS**	0.347 0.001 84	0.076 0.494 84	0.069 0.553 77	0.380 0.001 76	0.132 0.257 76
-0.262 0.020 84	0.168 0.139 84	-0.026 0.817 84	**DHEA7α**	0.488 0.000 84	0.035 0.761 77	0.230 0.045 76	0.034 0.768 76
-0.025 0.828 84	-0.153 0.179 84	0.009 0.938 84	0.477 0.000 84	**DHEA7β**	-0.035 0.762 77	0.122 0.296 76	0.048 0.678 76
-0.424 0.000 77	0.158 0.186 77	-0.234 0.047 77	-0.159 0.175 77	0.005 0.969 77	**TSH**	-0.085 0.468 76	-0.125 0.284 76
-0.041 0.735 76	-0.061 0.614 76	0.247 0.037 76	0.101 0.393 76	0.024 0.838 76	-0.062 0.600 76	**FT4**	0.435 0.000 76
-0.078 0.523 76	0.169 0.159 76	-0.147 0.219 76	-0.143 0.228 76	0.065 0.585 76	-0.164 0.160 76	0.403 0.000 76	**FT3**

Both 7-OH-DHEA isomers were increased by the treatment, and the between-stage differences revealed by ANOVA were highly significant. Maximum values of 7β-OH-DHEA were reached as early as during treatment (Stage 2), while those of 7α-OH-DHEA immediately after finishing the treatment (Stage 3). The rise of 7β-OH-DHEA was much more pronounced, being almost three times higher than the basal level. No significant changes were observed in DHEA/S levels.

As TSH and free thyroid hormones are concerned, in all instances the same trend was observed, namely a slight decrease of the levels during treatment (Day 5), followed by a small but significant rise, and then return again to the basal levels. An increase of TSH was not accompanied by opposite changes in thyroid hormone levels.

The basal data as well as those obtained at different stages of treatment (including those after termination of the administration) were mutually correlated. With respect to age differences of men, age was included as an additional parameter. Table 1 shows the correlation matrix for basal values, while Table 2 shows the correlation matrix for all the data obtained during and after treatment. The upper right parts from the diagonal represent simple paired values while the lower left parts the partial correlations. Correlation analysis was also performed separately for all stages of treatment (data not given), but they did not differ from those shown in Table 2.

Discussion

Early rise of 7β-OH-DHEA to values almost three times higher than those of 7α-OH-epimer during and after 7-oxo-DHEA application is in agreement with finding that the former is the prevailing reduced 7-oxo-DHEA metabolite (Robinzon *et al.* 2003). The fact that at least the levels of 7β-OH-DHEA still remained significantly increased one week after termination of 7-oxo-application may be considered advantageous with respect to its potentially beneficial effect.

The aim of correlation analysis was to reveal possible relations between DHEA/S and its 7-hydroxylated metabolites on one hand, and thyroid parameters on the another. Considering the basal levels, the only positive correlation found by both statistical approaches was between DHEAS and fT_4. This is in agreement with higher DHEA/S levels in hyperthyroidism and lower levels in hypothyroidism reported by Tagawa *et al.* (2000). When all the data during and after treatment were put together irrespective of the stage, the positive correlation was extended to unconjugated DHEA. There was, however, no relationship between any thyroid parameter and 7-OH-DHEA epimers, with only marginal positive correlation between 7α-OH-DHEA and fT_4 found by a simple pair method. In keeping with previous reports (Hampl *et al.* 2001), a significant decline of DHEA/S and its 7-hydroxylated metabolites was observed with age.

It was concluded that short-term treatment with 7-oxo-DHEA does not significantly affect the thyroid parameters, and that it provides considerable amounts of 7β-hydroxy-DHEA.

Acknowledgements

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