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Delayed effects of short-term transdermal application of 7-oxo-dehydroepiandrosterone on its metabolites, some hormonal steroids and relevant proteohormones in healthy male volunteers

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Abstract

Twenty-one healthy male volunteers aged 20–70 years were given transdermally 25 mg of 7-oxo-dehydroepiandrosterone daily in the form of an emulgel for 8 consecutive days. Morning blood was collected as follows: before application, and after the first, fourth and eighth doses (days 0, 2, 5 and 9), and then at different time intervals after termination of the treatment (days 16, 23, 37, 51, 72 and 100). Cortisol, testosterone, epitestosterone, estradiol, dehydroepiandrosterone and its sulfate, 7α- and 7β-hydroxy-dehydroepiandrosterone, luteinizing hormone, follicle-stimulating hormone and sex hormone-binding globulin were measured in blood sera. In the course of treatment 7β-hydroxy-dehydroepiandrosterone was significantly increased; testosterone and gonadotropins were lowered, but only after the first dose. All other significant changes were observed during the period after termination of the application: 7β-hydroxy-dehydroepiandrosterone remained increased for 28 days, 7α-hydroxy-dehydroepiandrosterone, testosterone, estradiol and sex hormone-binding globulin were decreased as late as day 63 and 91, respectively. On the other hand, epitestosterone was significantly increased between days 23 and 100. The levels of all other parameters studied were not significantly changed. The study points to an immediate as well as delayed effect of the short-term transdermal application of 7-oxo-dehydroepiandrosterone on relevant hormonal parameters.

Keywords: delayed effect; epitestosterone; 7-hydroxy-dehydroepiandrosterone; 7-oxo-dehydroepiandrosterone; transdermal application.

Introduction

Dehydroepiandrosterone (DHEA) is one of the most abundant products of the adrenal cortex and, to a lesser extent, of the gonads, and is one of the precursors in the biosynthetic pathways leading to testosterone and estrogens. In various human and animal tissues it is oxygenated on C7 to 7α-hydroxy-dehydroepiandrosterone (7α-OH-DHEA), 7β-hydroxy-dehydroepiandrosterone (7β-OH-DHEA) and 7-oxo-dehydroepiandrosterone (7-oxo-DHEA) (1–4), which may undergo mutual interconversion (4). 7-Hydroxylated metabolites of DHEA are believed to be responsible for at least some of its effects (4, 5). Recently, increased attention has been paid to 7-oxygenated 3β-hydroxysteroid derivatives: it was demonstrated that these metabolites are involved in various physiological processes (6–17) and may play a role in certain pathological states (18–20). In many of these functions 7-oxygenated metabolites were more efficient than their parent steroids (8, 13, 14).

To compare the effects of short-term transdermal application of DHEA and its 7-oxo-derivative, we carried out two pilot studies (21, 22). The levels of selected steroids, gonadotropins and lipids were followed after administration of DHEA and 7-oxo-DHEA in the form of an emulgel to 10 healthy males of different ages (21, 22). Surprisingly, DHEA administration significantly still influenced the levels of some hormonal parameters 5 weeks after finishing the treatment (21). The effects of 7-oxo-DHEA were followed only immediately after the last dose (22). We were interested as to whether transdermally applied 7-oxo-DHEA also still influences some hormonal parameters after a certain time interval. Therefore, we carried out a new study with an extended number of participants, who received 7-oxo-DHEA transdermally for 8 days. Serum concentrations of 7-hydroxylated DHEA metabolites, major sex steroids, gonadotropins and sex hormone-binding globulin (SHBG) were then measured before, during and immediately after termination of the treatment, and after intervals of several weeks after the last application. The results of this follow up are given here.

Materials and methods

Steroids and other chemicals

5-Androsten-3β-ol-7,17-dione (7-oxo-DHEA), 5-androstene-3β,7α-diol-17-one (7α-OH-DHEA), 5-androstene-3β,7βdiol-17-one (7β-OH-DHEA), 4-androsten-17β-ol-3-one (testosterone), 4-androsten-17α-ol-3-one (epitestosterone) and 4-pregnen-11β,17,21-triol-3,20-dione (cortisol) were purchased from Steraloids Inc (Newport, RI, USA). Diethyl ether and chemicals used for radioimmunoassay, all of analytical grade, were purchased from Merck (Darmstadt, Germany). Emulgel

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containing 0.5 g of 7-oxo-DHEA per 100 g was obtained from A. Nováček (Hilbertova 2, Ústí nad Labem, Czech Republic).

Subjects

The group of volunteers consisted of 21 informed healthy men aged 20–70 years (mean ± SD, 44.8 ± 14.4 years). Subjects were neither on regular medication nor had health risks, 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 treatment of successive groups was 2 weeks. The purpose of this arrangement was to avoid possible seasonal effects, as well as eventual fluctuation of analytical methods. The whole study thus lasted 10 months.

Treatment protocol

7-Oxo-DHEA was given transdermally as an emulgel. Approximately 5 g of the emulgel, corresponding to a daily dose of 25 mg of 7-oxo-DHEA, was applied before sleeping at 22:00 h on abdominal skin for 8 consecutive days. Morning blood collections were carried out after a night fast before the start of treatment (day 0), in the course of the treatment (days 2 and 5), on the day after the last application (day 9), and 1 week (day 16), 2 weeks (day 23), 4 weeks (day 37), 6 weeks (day 51), 9 weeks (day 72), and 13 weeks (day 100) after termination of the treatment. Blood sera were refrigerated and stored at −20°C until analysis.

Steroid determination

Serum cortisol (23), testosterone (24), epitestosterone (epiT, 17α-hydroxy-4-androstene-3-one) (25), 7α-hydroxy-DHEA (26) and 7β-hydroxy-DHEA (27) were determined by radioimmunoassay (RIA) using antisera and radioactive tracers prepared in the author's laboratory. Estradiol, DHEA, and DHEA sulfate (DHEAS) were determined by commercial RIA kits from Immunotech (Czech Division, Praha, Czech Republic). The free testosterone index (FTI) was calculated as the ratio testosterone/SHBG.

For determination of DHEA and its 7-hydroxylated metabolites, an extraction step followed by HPLC separation was included prior to RIA. Serum (2 mL) was extracted twice with 6 mL of diethyl ether. After evaporation, the dry residue of the extract was dissolved in 70 μL of methanol, and 25 μL of the solution was injected into a HPLC system, as described below. For determination of DHEAS, the aqueous phase remaining after ether extraction was evaporated in a vacuum centrifuge at 30°C, and the dry residue was reconstituted in water to the original volume and processed as described in the manufacturer's instruction for serum.

HPLC separation

HPLC binary high-pressure gradient elution was applied as follows. Mobile phase A, 15% acetonitrile in water with 100 mg/L of ammonium bicarbonate; mobile phase B, methanol; flow rate, 1 mL/min. Gradient: start–1 min, 0% B; 1–11 min, linear gradient from 55% to 65% B; 11–14 min 100% B; 14–20 min, 0% B; overall time, 20 min.

Standard solutions of 7β-OH-DHEA, 7α-OH-DHEA and DHEA at concentrations of 0.1 mg/mL were each injected and detected by UV at 205 nm to find optimal collection times for the fractions (the injection port and fraction collector were rinsed at least 10 times after application of the standard solution to avoid sample contamination). The retention times for individual steroids were as follows (retention time/collection window/dead time): 7β-OH-DHEA, 9.8/9.5–10.3/0.4; 7α-OH-DHEA, 11.6/11.1–11.9/+0.4; and DHEA, 15.6/15.4–16.2/+0.4. The fractions were collected automatically overnight in glass tubes, evaporated in a speed-vacuum centrifuge and the dry residues were dissolved in methanol (100 μL). The tubes were stoppered and kept until further analysis. Prior to analysis, the methanol was evaporated in a vacuum centrifuge.

Determination of proteohormones

Commercial immunoradiometric assay (IRMA) kits from Immunotech were used for determination of serum luteinizing hormone (LH), follicle-stimulating hormone (FSH) and SHBG.

Statistical analysis

The results were evaluated using a two-way analysis of variance (ANOVA) model with the stage (day of the experiment) as the first factor and the subject as the second factor. Special care was focused on data pretreatment with regard to non-Gaussian data distribution, heteroskedasicity (non-constant variance) and non-homogeneity. The data were power-transformed to attain minimum skewness of Studentized residuals. To eliminate the influence of outliers, only data with absolute Studentized values <2.5 were considered. ANOVA was accomplished using the statistical software Statgraphics Plus 5 (Manugistics, Rockville, MD, USA). ANOVA tests were followed by least significant difference (LSD) multiple comparisons to test the differences between individual stages of the experiment.

Results

7-Oxo-DHEA metabolites

The immediate metabolites of administered 7-oxo-DHEA are the reduction products of 7-oxo group, i.e., 7α- and 7β-epimers of 7-OH-DHEA. As demonstrated in Figure 1, which shows the time changes for selected steroids and hormones during and after 7-oxo-DHEA application, it is apparent that 7-oxo-DHEA was reduced mainly to 7β-hydroxy-DHEA, the level of which increased after 8 days of application by 160% when compared to the basal value ($p<0.0002$). The increase in 7β-hydroxy-DHEA level was still significant 4 weeks (day 37, $p<0.02$) after termination of the application. On the last days of the follow up, this level reached basal values. In contrast to 7β-hydroxy-DHEA, the level of its 7α-hydroxy epimer increased only insignificantly (by 28%) after 8 days of application, and in the following days it even decreased (Figure 1). By 9 weeks after termination of the application, the decline from the basal level reached statistical significance (by 63%, $p<0.05$).

Sex steroids

The levels of testosterone on average decreased after 7-oxo-DHEA application (Figure 1). A significant decrease was observed after the first dose and then after 2, 4 and 9 weeks, respectively.

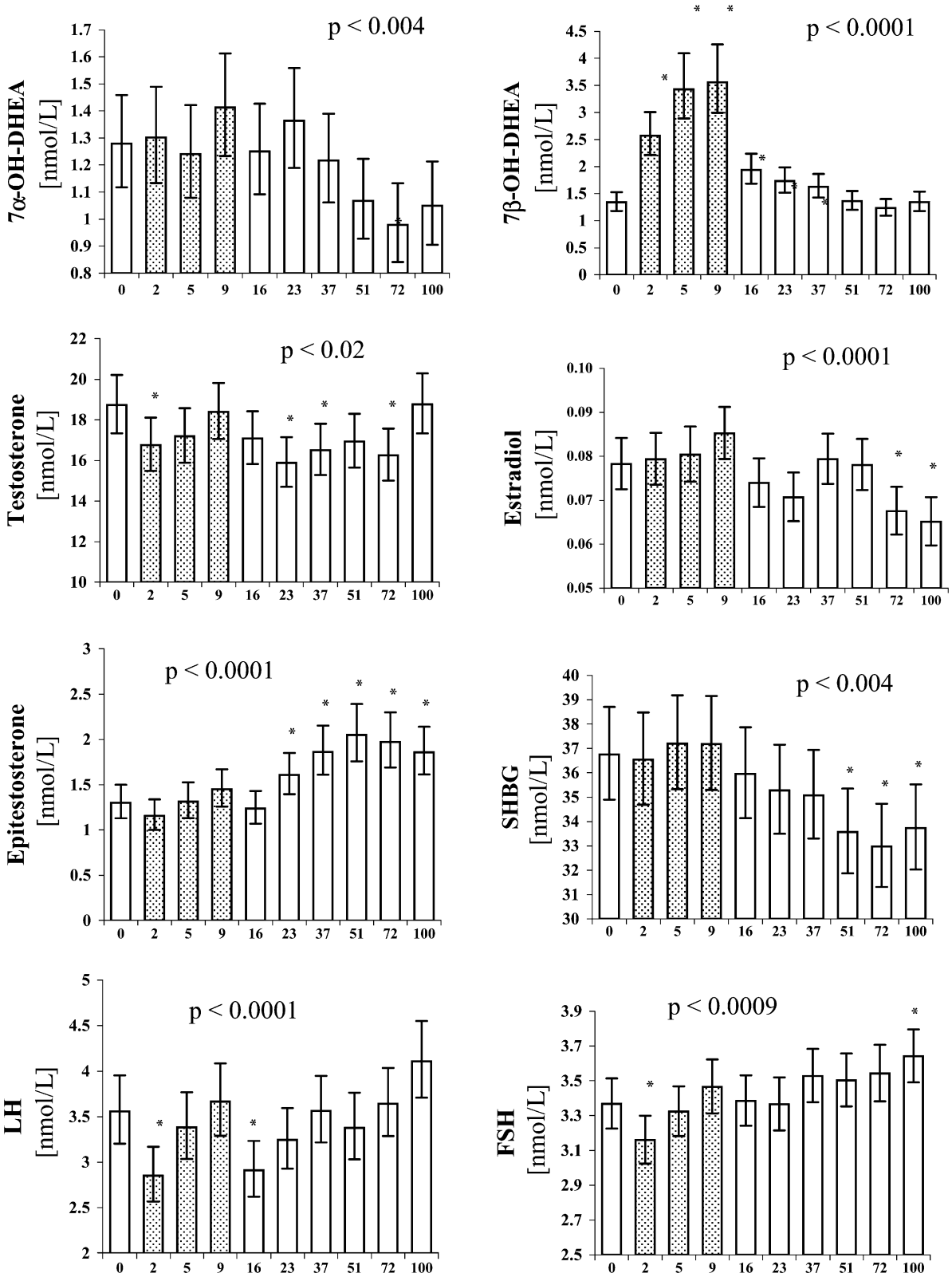

Figure 1 Levels of some steroids and proteohormones significantly changed after transdermal application of 7-oxo-DHEA to healthy men. The columns with error bars represent the retransformed mean values in individual stages of the experiment (days on the abscissa) with their 95% confidence intervals. The dotted columns symbolize 7-oxo-DHEA application. Asterisks represent the stages significantly different (p-values identified in Results) from basal levels as found by least significant difference multiple comparisons, with p-values designating the significance level of the repeated-measures ANOVA model.

The levels of estradiol did not change significantly during 7-oxo-DHEA administration, and even decreased during the following period. The decrease reached the level of statistical significance in the 9th and 13th weeks after termination of the application ($p < 0.05$), with a maximum of 48% with respect to the basal value (Figure 1).

In addition, the levels of epitestosterone did not change significantly during 7-oxo-DHEA administration, but from the 2nd week after termination of the application a significant increase was observed, which persisted until the 13th week. A maximum increase of 55% was reached 6 weeks after treatment ($p < 0.005$) (Figure 1).

Other steroids

No statistically significant changes were observed in the levels of cortisol, DHEA and DHEAS, neither during application nor in the following period (data not presented).

SHBG and FTI

The levels of SHBG showed great variation among participants, as is apparent from the large error bars in Figure 1. During application of 7-oxo-DHEA they did not change, but after its termination a successive decrease was observed, which reached the level of statistical significance from the 6th until the 13th week, with a maximum (by 55%, $p < 0.03$) 9 weeks after finishing the treatment.

The FTI was significantly decreased as early as after the first dose of 7-oxo-DHEA ($p < 0.05$) with respect to the basal value for 7-oxo-DHEA and also 23 days after termination of the application, similar to the total testosterone level. During the next period, FTI increased to levels close to basal values, reflecting the decrease in SHBG (data not presented).

Gonadotropins

LH and FSH responded to the first dose of 7-oxo-DHEA with a significant decrease in their levels (by 46% and 25%, respectively) (Figure 1). During further treatment they returned to basal levels. For both gonadotropins some other sporadic significant changes were recorded (see Figure 1). In general, the levels of both gonadotropins exhibited an increasing trend starting from the 2nd week after termination of treatment (Figure 1).

Discussion

In both sexes DHEA levels are strongly age-dependent (28, 29). They reach a peak in early adulthood and decline with increasing age (28, 29). This was one of reasons for using this adrenal androgen as a daily nutrition supplement for mitigation of aging (30, 31), and the effects of its administration have been extensively studied (32–40). In all these studies DHEA was administered perorally, but a percutaneous application was also tested in animals (41), as well as in postmenopausal women (42).

Recent reports (5, 7) demonstrated that not only DHEA itself, but its 7-hydroxylated metabolites may also be responsible for at least some immunoprotective, neuroprotective and thermogenic effects ascribed to DHEA. 7-Oxo-DHEA is another 7-oxygenated DHEA metabolite present in human blood, providing both 7-hydroxylated (7α- and 7β-) DHEA epimers, and it is also an intermediate of their interconversion (4). Its effects are similar to those of DHEA or its 7-hydroxylated metabolites. 7-Oxo DHEA positively influenced immunoreactivity in mice exposed to mild chronic stress (12). A single peroral dose of 7-oxo-DHEA acetate had a beneficial effect on cognitive functions in old mice (16). It was also demonstrated that 7-oxo-DHEA is an ergosteroid, capable of inducing thermogenic enzymes in the liver (14, 15).

Similar to DHEA, 7-oxo-DHEA was also administered perorally not only to animals (12, 14–16, 43, 44), but also to humans (45–47). When given to healthy men in the acetate form (3β-acetyl-7-oxo-DHEA) (45), it was rapidly converted to the sulfate, which is not accumulated in the organism. In blood collected a few hours after the last dose, concentrations of hormones were not affected by the treatment (45). This compound was well tolerated and therefore it was tested as a possible weight-reducing agent in otherwise healthy adults who were overweight (46). For the same purpose it was used as a component of the dietary supplement 7-Keto Naturalean™ (47). The question is: how would 7-oxo-DHEA be metabolized when applied in an unesterified form and what would be its effect(s) during treatment and at certain intervals after its termination? The chemical form and chronological factors in application of the steroid may play an important role.

In our two pilot studies (22, 48) 7-oxo-DHEA in the form of emulgel was applied percutaneously for a short time to a smaller number of healthy men. We found that when applied in this way, 7-oxo-DHEA was metabolized more to 7β- than to 7α-OH-DHEA (48), and that it significantly influenced the levels of some hormones (testosterone, FTI, estradiol, LH) when measured immediately after termination of the application (22). In another study with transdermally applied DHEA (21) we observed that the levels of some steroids (DHEA, testosterone, estradiol, androstenedione) and gonadotropins (LH) still differed from basal levels 5 weeks after finishing the treatment (21). Recently Kroboth et al. (40) studied the effect of DHEA administration to elderly women and men on plasma cortisol. In women they found a significant decrease in mean daily cortisol concentration not only during DHEA administration, but also 1 week after finishing the treatment (39).

In this 100-day study, 7-oxo-DHEA was given transdermally to 21 healthy men for 8 days. Serum levels of 7-hydroxylated DHEA metabolites, six other steroid parameters, gonadotropins and SHBG were measured. After ANOVA tests, significant changes were found in the mean levels of both 7α- and 7β-OH-

DHEA, testosterone, estradiol, epitestosterone, SHBG, LH and FSH. In accordance with the previous study (48) 7-oxo-DHEA was predominantly metabolized to the 7β-OH epimer, levels of which were still significantly increased 6 weeks after finishing the treatment. It is also in agreement with results of in vitro experiments (4) in which human liver microsomal fractions reduced 7-oxo-DHEA mainly to 7β-OH-DHEA (4). This could be of practical importance. Considering the beneficial effects of 7β-OH-DHEA (9), in particular neuroprotective effects, 7-oxo-DHEA may be a drug of choice for steroid replacement therapy, for example, in Alzheimer's dementia (AD). Recently, Kim et al. (20), when analyzing cerebrospinal fluid (CSF) for selected DHEA metabolites, pointed to a relative lack of 7β-hydroxylation in the brains of patients with AD. The 7β-OH-DHEA/DHEA ratio in CSF was significantly lower than in control subjects (20). Because 7-oxo-DHEA appeared to be an ample source of 7β-OH-DHEA, it could serve for prevention of AD better than DHEA itself. Taking into account the relatively low activity of 7β-hydroxylase (20), one condition for its functioning is sufficient activity of 7β-hydroxysteroid dehydrogenase (7β-HSD) in AD. The latter enzyme has been proven in in vitro experiments in both humans and rodents (49, 50), and further characterized in rabbit liver microsomes (52). It was classified as a member of the type 1 11β-hydroxysteroid dehydrogenase (11β-HSD) family, and its dual activity was demonstrated (51, 52).

In our present study, in contrast to 7β-OH-DHEA, the levels of 7α-OH-DHEA increased during treatment only insignificantly, and starting from the 6th week they declined to a value significantly lower than the basal level. For the eventual use of 7-oxo-DHEA in treatment, if it is definitively proven that 7α-OH-DHEA positively influences some physiological processes described previously in animals, especially those also concerning the immune system in humans (6–8, 10, 11, 13), then percutaneously administered 7-oxo-DHEA may not be a suitable agent.

Testosterone response to transdermal treatment of men with 7-oxo-DHEA tended towards a decline, which, however, reached a significant level as late as 2 weeks after termination of the application. This is not fully in agreement with the results of our pilot study, in which a decline occurred immediately after 5 days of application (22). Simultaneously with decreasing testosterone, the levels of its 17α-OH epimer (epitestosterone) significantly increased within days 23–72 of the study. With respect to the fact that antiandrogenic effects are ascribed to epitestosterone (51, 52), there is the possibility of diminishing total androgenic potential after transdermal application of 7-oxo-DHEA. After a longer interval after treatment, this effect could be partially compensated by a concomitant significant decrease in SHBG levels, found in the 6th week after termination of steroid treatment, and of estradiol levels (from the 9th week on).

Generally, the gonadotropins, in spite of fluctuations in their levels, displayed a tendency to increase after termination of the treatment, in agreement with the opposite trend observed in sex hormones.

Although the protracted effect of transdermally administered 7-oxo-DHEA could to some extent be expected, taking into account the results of our pilot study with DHEA (21), some results from the new study were surprising. The persistent significantly increased levels of the main metabolite 7β-OH-DHEA 4 weeks after termination of treatment were unexpected. This may be related to delayed effects of major (testosterone, estradiol, SHBG) as well as less common (epiT, 7α-OH-DHEA) hormonal parameters.

To the best of our knowledge, there is no such observation available, and it is difficult to make a definite conclusion based on a single study. We would like to point out the phenomenon of long-term or delayed effect(s) of steroid drugs. The delayed effects described here may be specific for percutaneous application in the form of an emulgel, or they may be inherent only to compounds administered in unconjugated chemical form. Therefore, the importance of the application form with respect to persistent or delayed effects of steroids should be one of the first points to be addressed when considering steroid replacement therapy.

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