

Bioavailability and metabolism of oral and percutaneous dehydroepiandrosterone in postmenopausal women

F. Labrie*, A. Bélanger, C. Labrie, B. Candas, L. Cusan, J.L. Gomez

Molecular Endocrinology and Oncology Research Center, Laval University Hospital (CHUL) and Laval University, Quebec City, Quebec G1V 4G2, Canada

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Abstract

To study the bioavailability of dehydroepiandrosterone (DHEA) administered by the oral and percutaneous routes, three groups of 12 postmenopausal women aged 60–70 years received two capsules of 50 mg of DHEA orally before breakfast daily for 14 days or applied 4 g of a 10% DHEA cream or gel at the same time of the day on a 30 cm × 30 cm surface area on the thighs. Detailed serial blood sampling over 24 h was performed following 1st and 14th DHEA administration for measurement of DHEA and nine of its metabolites by liquid chromatography tandem mass spectrometry (LC–MS/MS) or gas chromatography mass spectrometry (GC–MS). Serum levels of estrone (E1) and estradiol (E2) did not change following DHEA administration by any of the three formulations, while serum androstenedione (4-dione), testosterone, DHEA sulfate (DHEA-S), E₁-S, androsterone glucuronide (ADT-G) and 3 α -androstenediol-G (3 α -diol-G), increased in all cases, the effect on these parameters being more important after oral than percutaneous administration due to the metabolism of DHEA into these metabolites in the gastrointestinal tract and liver. No qualitative differences in DHEA metabolism are observed between the oral and percutaneous routes of DHEA administration while the levels of all steroids remain on a plateau during the 24 h period during chronic percutaneous DHEA administration. The present data show that DHEA is transformed into active androgens and estrogens in peripheral intracrine tissues with no or minimal release of the active steroids E₁, E₂ or testosterone in the circulation. Moreover, DHEA is preferentially transformed into androgens rather than into estrogens. Most importantly, the present data show that changes in serum DHEA following oral or percutaneous DHEA administration are not a valid parameter of DHEA action since the increase in serum DHEA is at least 100% greater than the increase in the formation of active androgens and estrogens and thus much higher than the potential physiological effects.

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1. Introduction

Although dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S) were first isolated and identified in 1934 [1] and 1944 [2], respectively, and DHEA-S was isolated from human plasma in 1954 [3], the role of DHEA and DHEA-S in human endocrine physiology and disease is of relatively recent interest [4–7].

Humans, along with the other primates, are unique among animal species in having adrenals that secrete large amounts of the inactive precursor steroids DHEA and especially DHEA-S, which are converted into active androgens and/or estrogens in peripheral tissues [5,8–13]. In fact, plasma DHEA-S levels in adult men and women are 100–500 times higher than those of testosterone and 1000–10,000 times higher than those of estradiol, thus providing a large reservoir of substrate for conversion into androgens and/or estrogens in the peripheral intracrine tissues which possess the enzymatic machinery necessary to transform DHEA into active sex steroids [5,12]. In fact, the term intracrinology was first coined in 1988 [14] to describe the synthesis of the active steroids made in the same cells where they exert their action

* Corresponding author at: Oncology and Molecular Endocrinology Research Center, Laval University Medical Center (CHUL), 2705 Laurier Boulevard, Quebec City, Quebec G1V 4G2, Canada. Tel.: +1 418 654 2704; fax: +1 418 654 2735.

E-mail address: fernand.labrie@crchul.ulaval.ca (F. Labrie).

with no or minimal release into the extracellular space and general circulation before being inactivated [5].

The marked reduction in the formation of DHEA-S by the adrenals during aging [6,15,16] results in a dramatic fall in the formation of androgens and estrogens in peripheral target tissues, a situation potentially associated with age-related diseases such as insulin resistance [17,18] and obesity [19–21]. Moreover, much attention has been given to the benefits of DHEA administered to postmenopausal women, especially on the bone, skin, vagina, glucose and insulin metabolism, fat mass, as well as well-being after oral [7,22–24] and percutaneous [25,26] administration. It thus becomes of particular importance to obtain more precise knowledge about the bioavailability, pharmacokinetics and metabolism of DHEA following these two routes of administration.

Since we have already shown, using a pharmacological dose of DHEA administered percutaneously for 2 weeks, that measurements of serum testosterone (testo) and estradiol (E_2) levels do not provide a reliable assessment of the true intracellular pool of androgens and estrogens [27–29], we have compared the serum levels of DHEA and nine steroids known to be most closely associated with active androgens and estrogens and their metabolites. A detailed analysis of the 24 h changes of serum steroid levels was performed on the first day and after 2 weeks of daily administration of DHEA by the oral route as well as percutaneously using a DHEA cream or gel.

2. Subjects and methods

Thirty-six healthy 60–70-year-old postmenopausal women participated in the study after IRB approval and having given their written informed consent. Body weight was within $\pm 20\%$ of normal body weight according to Metropolitan Life Tables.

No subject suffered from a significant metabolic or endocrine disorder, coronarian disease or hypertension. No women had treatment with androgens or anabolic steroids within 6 months prior to the screening visit. All participants had a medical history, complete physical examination and serum biochemistry profile including lipids, complete blood count, urine analysis and detailed serum hormone determinations during the screening phase of the protocol.

3. Study design, treatment and measurements

This study was a randomized open-label trial of 12 subjects per arm. After written informed consent was obtained and women were found eligible, each subject was randomized to receive DHEA by cream, gel or orally. Daily, before breakfast, for 14 days, subjects received, at the research clinic, either 4 g of 10% DHEA gel or 4 g of 10% DHEA cream applied on a total 30 cm \times 30 cm area of the thighs or two 50 mg capsules of DHEA orally before breakfast.

Blood sampling was performed at 08:00–09:00 h at screening and before application of DHEA, on the first day of dosing, as well as on days 2, 4, 7, 10 and 14. On the 1st and 14th days, blood samples were obtained at 0.5 h, 1 h, 1.5 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 12 h and 24 h following DHEA administration.

4. Serum steroid analysis

DHEA, DHEA-S, androst-5-ene-3 β ,17 β -diol (5-diol), testosterone, androstenedione (4-dione), 17 β -estradiol (E_2), estrone (E_1), estrone sulfate (E_1 -S), androsterone glucuronide (ADT-G), and androstane-3 α ,17 β -diol glucuronide (3 α -diol-G) were measured by gas chromatography/mass spectrometry (DHEA, 5-diol, 4-dione, testosterone, E_1 and E_2) using electron impact or chemical ionization and by liquid chromatography/tandem mass spectrometry using turboion-spray (DHEA-S, E_1 -S, ADT-G and 3 α -diol-G) as described [28–30].

5. Calculations and statistical analysis

On days 1 and 14, the area under the curve of the serum concentration of each steroid was measured between 0 h and 24 h (AUC_{0–24h}). The areas under the curves were calculated by a linear trapezoidal method (model-independent). The relative bioavailability of the DHEA gel, DHEA cream and DHEA capsules was based on the mean difference in the log-transformed AUC values. All calculations were performed with the SAS software (SAS Institute, Cary, NC, USA).

6. Results

The oral administration of two capsules of 50 mg of DHEA led to an increase of serum DHEA from 2.3 ± 0.3 ng/ml to a maximal value of 15.6 ± 2.5 ng/ml at 1 h with a progressive decrease thereafter to 5.7 ± 0.5 ng/ml at 6 h followed by a plateau up to 24 h (Fig. 1A). When 4 g of a 10% DHEA gel or cream were applied on a 30 cm \times 30 cm area of the skin of the thighs, serum DHEA levels only started to increase at 12 h to reach values of 8.2 ± 2.0 and 8.0 ± 1.2 nmol/l, respectively, at 24 h (Fig. 1A). There was no significant difference between the cream or gel in the serum levels of DHEA at any of the time intervals studied up to 24 h after first application of the precursor steroid on the skin.

When serum 5-diol was measured after oral first administration of DHEA, the concentration of 5-diol increased from a pretreatment concentration of 0.31 ± 0.03 ng/ml to a maximal value of 1.19 ± 0.13 ng/ml at 1 h with a slow and progressive decrease thereafter to reach 0.79 ± 0.05 ng/ml at 24 h (Fig. 1B). It can be seen in the same figure that the serum levels of 5-diol increased much more slowly after administration of DHEA percutaneously by cream or gel to reach the first

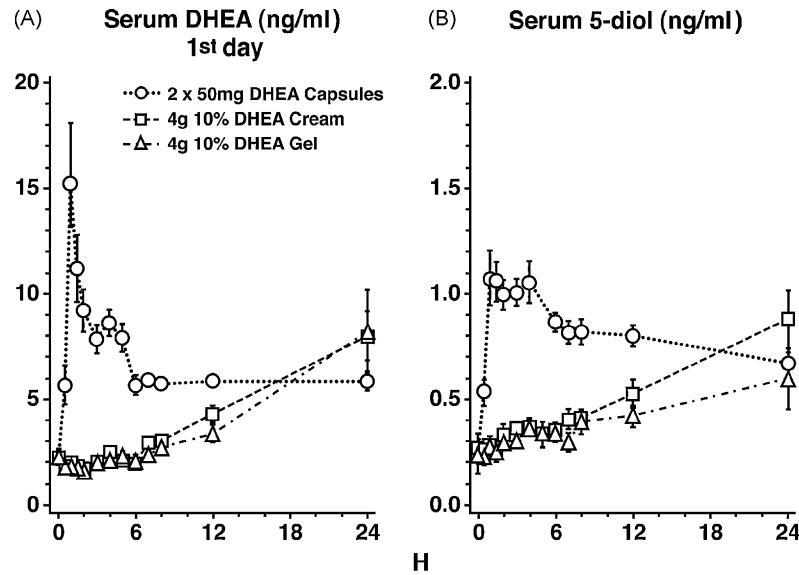


Fig. 1. Time-course of serum dehydroepiandrosterone (DHEA) (A) and androst-5-ene-3 β ,17 β -diol (5-diol) (B) following single oral administration of two 50-mg capsules of DHEA or the application of 4 g of 10% DHEA cream or gel to postmenopausal women.

statistically significant different values of 1.00 ± 0.14 ng/ml for the cream and 0.72 ± 0.14 ng/ml for the gel at 24 h.

Following oral DHEA, serum 4-dione increased from 0.6 ± 0.1 ng/ml to a maximal value of 9.5 ± 2.2 ng/ml at 1 h followed by a rapid decrease thereafter to values which remained on a plateau of about 1.2 ng/ml between 8 h and 24 h (Fig. 2A). Following administration of DHEA by cream or gel, on the other hand, the first significant increase of serum 4-dione was only observed at 24 h at values of 0.9 ± 0.1 and 0.8 ± 0.1 ng/ml for the cream and gel, respectively.

A comparable pattern was observed for serum testosterone. In fact, after oral administration of two 50 mg capsules of DHEA, serum testosterone increased from

0.38 ± 0.03 ng/ml to a maximal value of 0.79 ± 0.14 ng/ml at 1 h. This rise was followed by a rapid decrease to 0.30 ± 0.08 ng/ml at 6 h followed by a plateau thereafter until 24 h (Fig. 2B). When DHEA was applied as cream or gel, the first increase was observed at 24 h at a value of approximately 0.45 ng/ml. As can be seen in Fig. 3A and B, the first administration of DHEA by the oral or percutaneous route had no statistically significant effect on the serum levels of E1 or E2 during the first 24 h.

Serum DHEA-S on the other hand followed a pattern similar, although slightly delayed, compared to DHEA and 5-diol following oral administration of two capsules of 50 mg DHEA (Fig. 4A). Thus, serum DHEA-S increased from

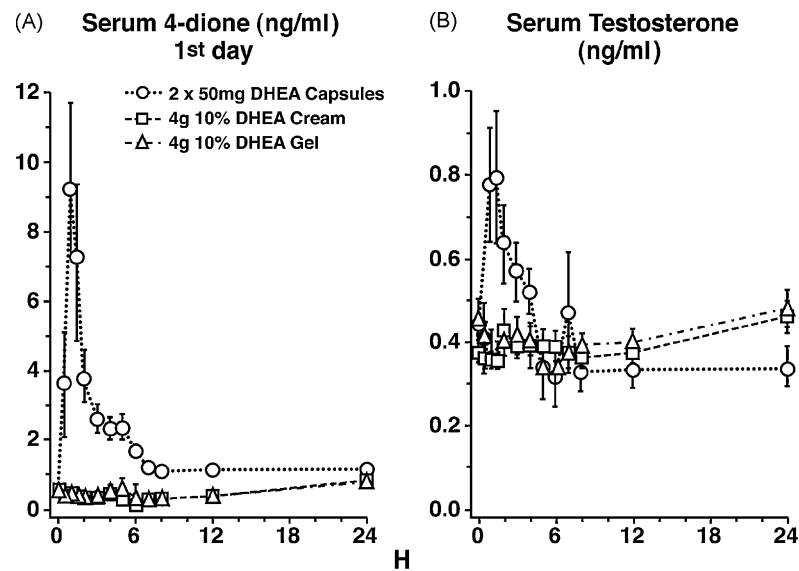


Fig. 2. Time-course of serum androstenedione (4-dione) (A) and testosterone (B) following single oral administration of two 50-mg capsules of DHEA or the application of 4 g of 10% DHEA cream or gel to postmenopausal women.

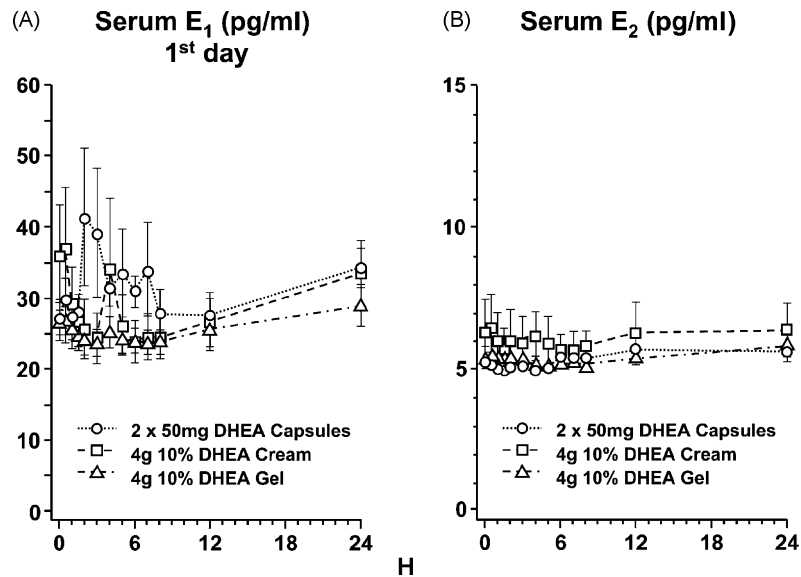


Fig. 3. Time-course of serum estrone (E₁) (A) and 17β-estradiol (E₂) (B) following single oral administration of two 50-mg capsules of DHEA or the application of 4 g of 10% DHEA cream or gel to postmenopausal women.

0.4 ± 0.1 μg/ml to 7.7 ± 1.0 μg/ml at 1 h to a maximal value of 8.4 ± 0.6 μg/ml at 2 h with a progressive decrease to 2.7 ± 0.3 μg/ml at 24 h. No significant change of serum DHEA-S was observed during the first 24 h after administration of DHEA in a cream or gel. Serum E₁-S, on the other hand, did not change significantly during the first 24 h following the first administration of DHEA by any of the three routes of administration (Fig. 4B).

Serum ADT-G, the main metabolite of androgens, increased from 14 ± 3 ng/ml to 760 ± 150 ng/ml at 1 h and 790 ± 140 ng/ml at 2 h to then decrease progressively to 92 ± 5 ng/ml at 12 h and 70 ± 5 ng/ml at 24 h (Fig. 5A). Serum 3α-diol-G, on the other hand, increased from

2.2 ± 0.5 ng/ml to 14.5 ± 2.0 ng/ml at 2 h (Fig. 5B). The decrease observed thereafter for 3α-diol-G was however much slower than that of ADT-G, a decrease of only about 40% being observed between 2 h and 24 h after oral administration of DHEA. Following application of 4 g of 10% DHEA on the skin, there was no significant change of serum ADT-G or 3α-diol-G up to 24 h (Fig. 5B).

When the measurements of the same kinetic parameters were repeated on the 14th day of daily dosing, it could be seen that the administration of two capsules of 50 mg of DHEA led, from a predosing value of 4.2 ± 0.4 ng/ml, to a maximal concentration of 14.8 ± 4.4 ng DHEA/ml at 1 h followed by a progressive decrease thereafter to 4.5 ± 0.4 ng/ml at 24 h

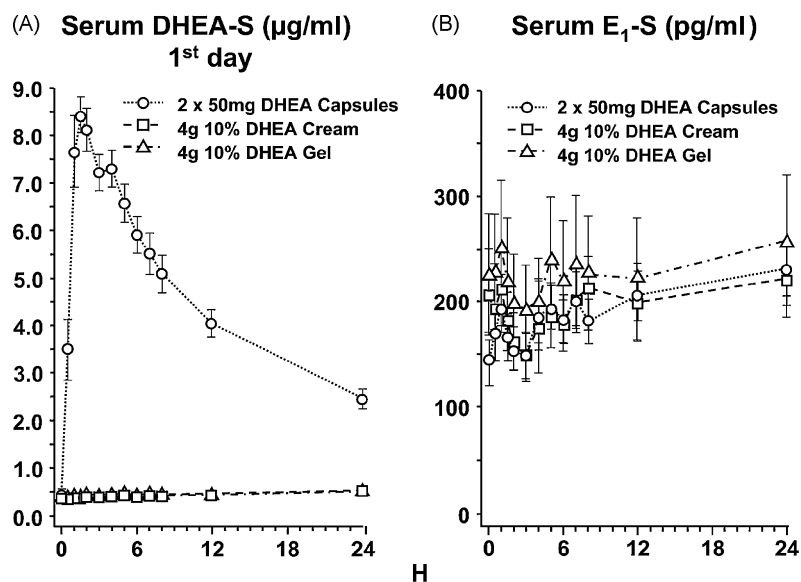


Fig. 4. Time-course of serum dehydroepiandrosterone sulfate (DHEA-S) (A) and estrone sulfate (E₁-S) (B) following single oral administration of two 50-mg capsules of DHEA or the application of 4 g of 10% DHEA cream or gel to postmenopausal women.

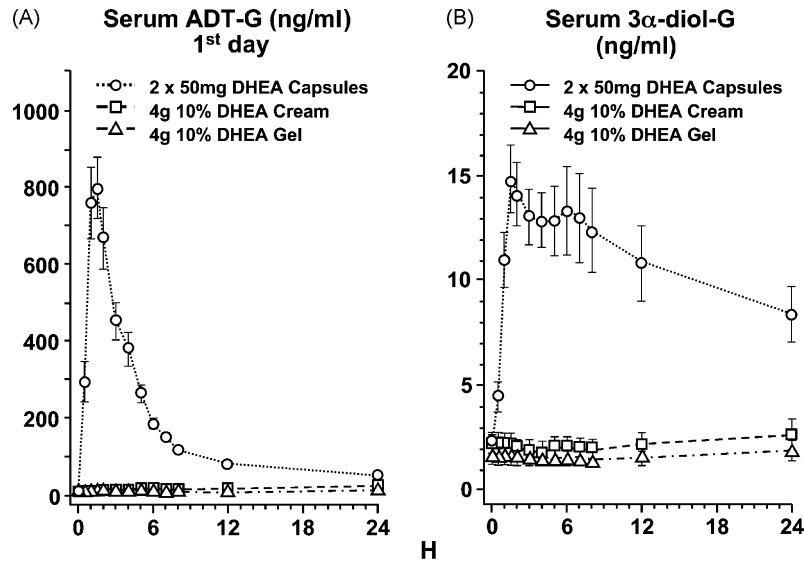


Fig. 5. Time-course of serum androsterone glucuronide (ADT-G) (A) and androstane 3 α ,17 β -diol-glucuronide (3 α -diol-G) (B) following daily oral administration of two 50-mg capsules of DHEA or the application of 4 g of 10% DHEA cream or gel to postmenopausal women.

(Fig. 6A). On the other hand, when DHEA was administered by cream or gel, no significant change was observed during the 24-h period and serum DHEA remained between 10 ng/ml and 15 ng/ml following application of the cream and between 7 ng/ml and 11 ng/ml following application of the gel.

Similarly, when serum 5-diol was measured on the 14th day of treatment, the serum concentration of this steroid increased from 0.46 ± 0.04 ng/ml to 1.37 ± 0.21 ng/ml at 1 h with a slow decrease thereafter to reach 0.64 ± 0.06 ng/ml at 24 h (Fig. 6B). As observed for DHEA, serum 5-diol remained approximately constant during the 24-h period at about 1.5–1.9 ng/ml following application of the cream and 1.0–1.3 ng/ml following application of the gel.

When serum 4-dione was measured on the 14th day of dosing, the serum concentration of this steroid increased from 1.3 ± 0.2 ng/ml to a maximal value of 9.8 ± 1.7 ng/ml at 1 h followed by a rapid decrease to 1.5 ± 0.1 ng/ml at 6 h with a value of 1.2 ± 0.1 ng/ml measured at 24 h (Fig. 7A). Following application of DHEA on the skin as a cream or gel, there was a non-significant increase of serum 4-dione to approximately 2.5 ng/ml at 2 h with values, thereafter, remaining on a plateau at 1.0–1.6 ng/ml up to 24 h (Fig. 7A).

Serum testosterone increased on the 14th day of dosing following oral administration of 100 mg of DHEA from 0.31 ± 0.04 ng/ml to a maximal value of 0.83 ± 0.11 ng/ml at 1 h followed by a progressive decrease to a value of

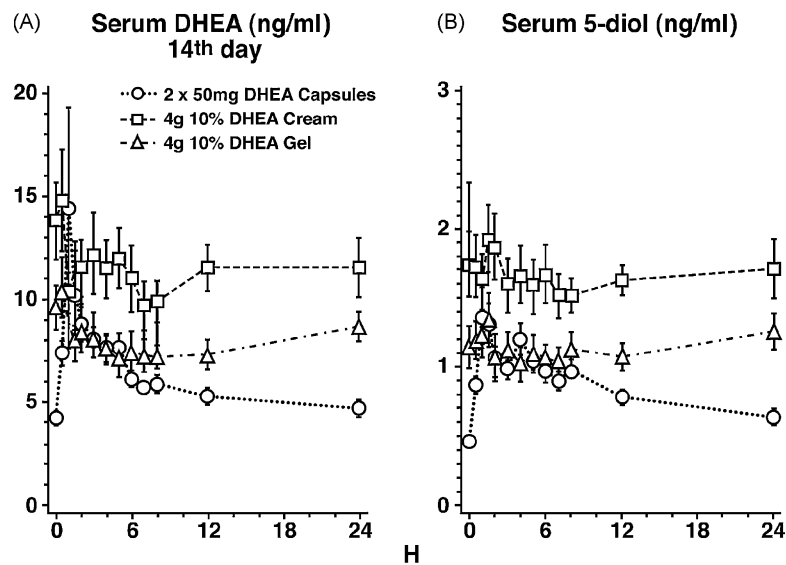


Fig. 6. Time-course of serum dehydroepiandrosterone (DHEA) (A) and andros-5-ene-3 β ,17 β -diol (5-diol) (B) following daily oral administration of two 50-mg capsules of DHEA or the application of 4 g of 10% DHEA cream or gel to postmenopausal women. Measurements were made on the 14th day of dosing.

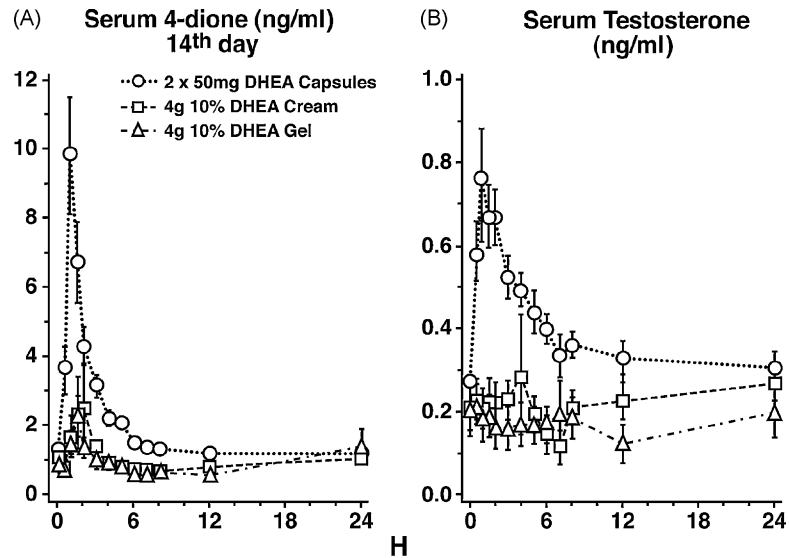


Fig. 7. Time-course of serum androstenedione (4-dione) (A) and testosterone (B) following daily oral administration of two 50-mg capsules of DHEA or the application of 4 g of 10% DHEA cream or gel to postmenopausal women. Measurements were made on the 14th day of dosing.

0.37 ± 0.04 ng/ml at 24 h (Fig. 7B). Following DHEA application as a cream or a gel, serum levels of testosterone remained unchanged during the 24-h period at approximately 0.3 ng/ml, this value being not significantly different from pretreatment. As observed on the first day, there was no significant change in the serum levels of E1 (Fig. 8A) or E2 (Fig. 8B) during the 24 h which followed the 14th daily administration of DHEA by the oral or percutaneous route.

From a predosing level of 1.95 ± 0.15 μ g/ml, serum DHEA-S increased to 8.3 ± 0.4 μ g/ml at 1 h to decrease progressively to 2.6 ± 0.3 μ g/ml at 24 h (Fig. 9A). No significant change in serum DHEA-S was observed after application

of DHEA on the skin. Serum E₁-S, on the other hand, did not change during the 24 h following the 14th daily administration of DHEA by the oral or percutaneous route (Fig. 9B).

While starting at a higher level on day 14 than on day 1, serum ADT-G increased rapidly from 66 ± 1 ng/ml to 996 ± 105 ng/ml at 1 h to decrease progressively thereafter to 116 ng/ml at 12 h and 91 ± 15 ng/ml at 24 h (Fig. 10A). No significant change in serum ADT-G levels occurred following the application of DHEA on the skin. Serum 3 α -diol-G, on the other hand, increased from 12 ± 2.5 ng/ml to 29.4 ± 5.5 ng/ml at 2 h to decrease slowly thereafter to reach

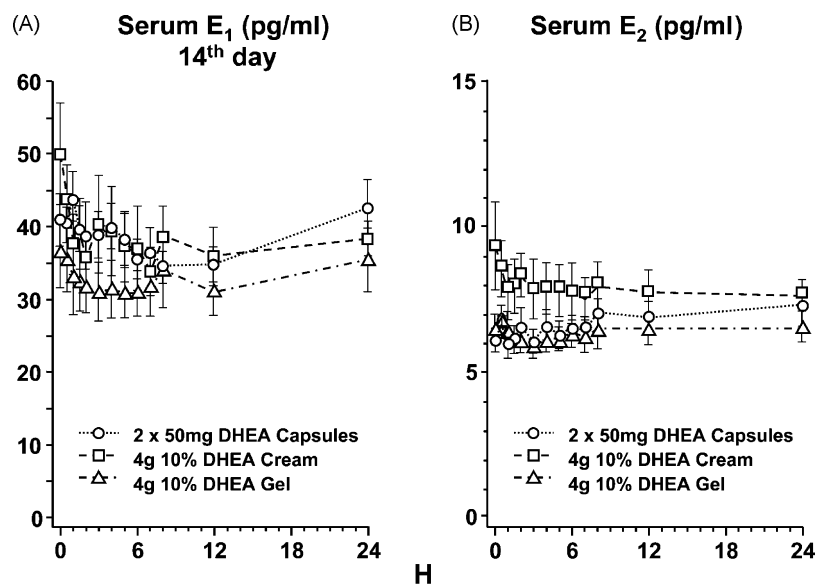


Fig. 8. Time-course of serum estrone (E₁) (A) and estradiol (E₂) following daily oral administration of two 50-mg capsules of DHEA or the application of 4 g of 10% DHEA cream or gel to postmenopausal women. Measurements were made on the 14th day of dosing.

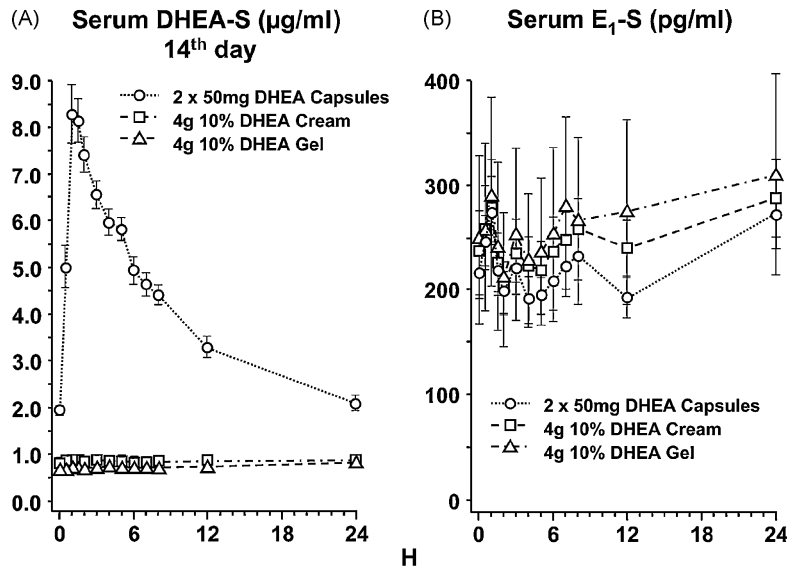


Fig. 9. Time-course of serum dehydroepiandrosterone sulfate (DHEA-S) (A) and estrone sulfate (E₁-S) (B) following daily oral administration of two 50-mg capsules of DHEA or the application of 4 g of 10% DHEA cream or gel to postmenopausal women. Measurements were made on the 14th day of dosing.

13 ± 3.0 ng/ml at 24 h following 14th daily oral administration of 100 mg DHEA. No significant change was observed on serum 3α-diol-G after percutaneous administration of DHEA (Fig. 10B).

In order to obtain a more precise measure of the accumulation of DHEA and its metabolites, we next compared the areas under the curves of the serum steroid concentrations (AUC_{0-24h} values) measured on the 1st and 14th days of dosing. As can be predicted from Figs. 1–10, the AUC_{0-24h} values of all steroids, except the metabolites of estrogens (E₁-S) and androgens (ADT-G and 3α-diol-G), due to some accumulation of these steroids, are similar on the 1st and 14th

days of administration of DHEA by the oral route (Table 1). Following percutaneous administration of DHEA, on the other hand, due to the slower absorption of DHEA following administration in a cream or gel, 155% and 86% higher values of the DHEA AUC_{0-24h} values are observed on the 14th day compared to the first day of dosing, respectively. Higher values are also observed for all the other steroids, except for E₁, E₂ and testosterone which showed no significant change after DHEA administration.

As can be clearly seen in Table 2 and Fig. 11, there was no significant change in the serum E₁, E₂ or testosterone AUC_{0-24h} values measured on the 14th day of dosing com-

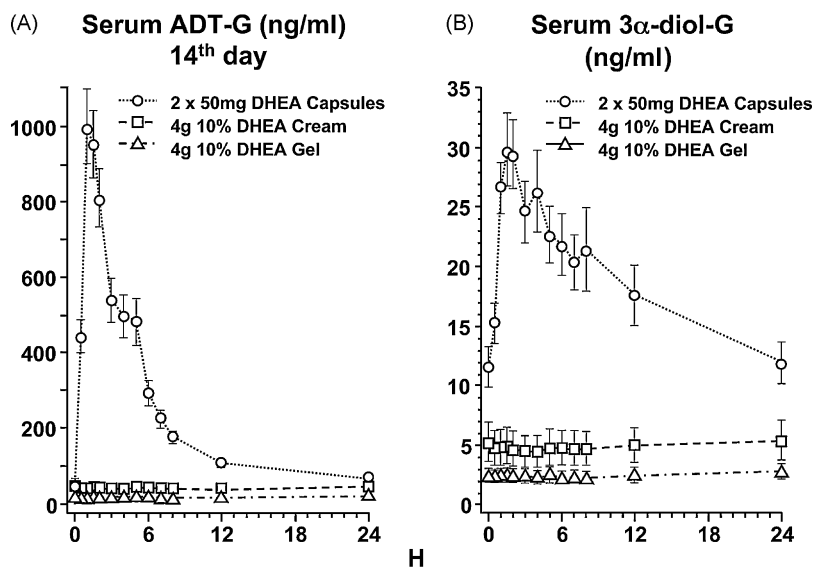


Fig. 10. Time-course of serum androsterone glucuronide (ADT-G) (A) and androstene-3α,17β-diol-G (3α-diol-G) (B) following daily oral administration of two 50-mg capsules of DHEA or the application of 4 g of 10% DHEA cream or gel to postmenopausal women. Measurements were made on the 14th day of dosing.

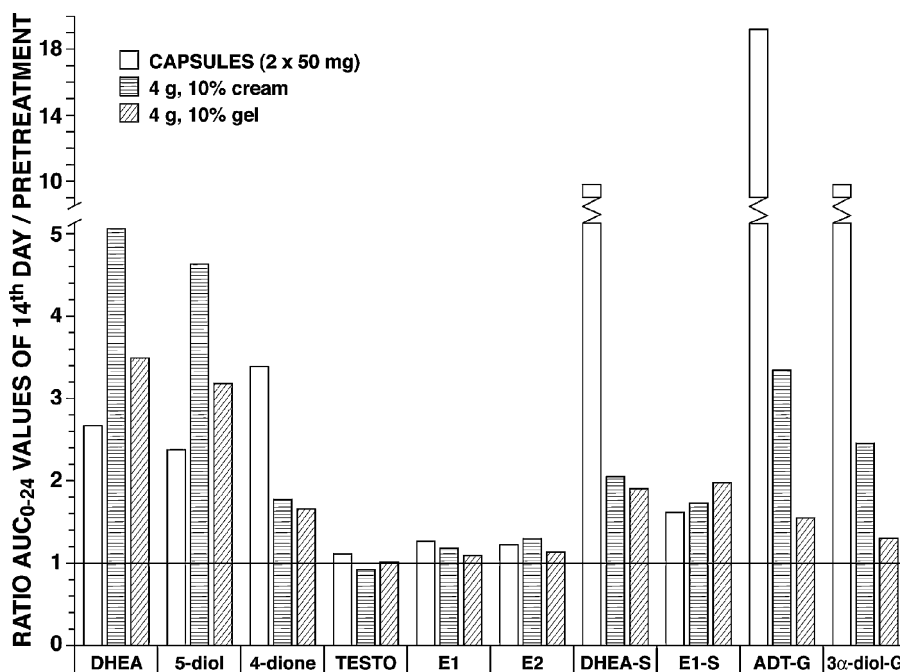


Fig. 11. Ratios of the AUC_{0-24h} values of DHEA and its metabolites on the 14th day of dosing compared to the pretreatment basal values. The corresponding numerical values can be found in Table 2.

pared to the predosing levels. Significant increases, however, were observed for all two other steroids. Thus, following daily oral dosing with 100 mg DHEA for 2 weeks, the area under the concentration curve of DHEA measured during the 24 h following administration of the steroid increased 167% over the pretreatment value while for 5-diol, 4-dione, DHEA-S, E₁-S, ADT-G and 3α-diol-G, respective increases of 138%, 238%, 873%, 60%, 1820% and 874% were observed.

Except for DHEA and 5-diol, lower increases were observed following administration of DHEA cream or gel. In fact, following application of the DHEA cream, the DHEA-S AUC_{0-24h} value increased by only 104% while the 4-dione, E₁-S, ADT-G and 3α-diol-G AUC_{0-24h} values increased by 77%, 72%, 234% and 145% over control, respectively. The AUC values for DHEA and 5-diol, on the other hand, increased by 406% and 363%, respectively (Table 2, Fig. 11). Comparable but somewhat lower increases were observed with the DHEA gel where the serum 4-dione, DHEA-S, E₁-S, ADT-G and 3α-diol-G AUC_{0-24h} values increased by 65%, 91%, 96%, 56% and 30% over control while the AUC_{0-24h} values for DHEA and 5-diol increased by 249% and 238%, respectively.

Our recent findings [29] have shown that the serum DHEA changes observed following exogenous DHEA administration are at least a 100% overestimate of the true changes in sex steroid formation. In support of these data, Fig. 11 shows that following DHEA administration by cream or gel, the changes in serum DHEA are a marked overestimate of the changes in serum levels of all the steroids measured except for 5-diol, the immediate metabolite of DHEA. For the DHEA cream, the changes in the AUC_{0-24h} values of serum 4-dione,

DHEA-S, E₁-S, ADT-G and 3α-diol-G are only 77%, 104%, 72%, 234% and 145% compared to the 406% increase over pretreatment levels observed for serum DHEA.

For the androgens, it is now well established that uridine glucuronosyl transferase 2B7 (UGT 2B7), UGT 2B15 and UGT 2B17 are the three enzymes responsible for the glucuronidation of all androgens and their metabolites in the human [31]. This recent completion of the identification and characterization of all the human UDT-glucuronosyl transferases makes possible the use of the glucuronide derivatives of androgens as markers of total androgenic activity in both women and men [28–30]. Accordingly, since all androgens are metabolized into ADT-G and 3α-diol-G, the estimate of the percentage of efficacy of percutaneous DHEA for transformation into active androgens is thus estimated at 52% when adding the changes in ADT-G and 3α-diol-G (a weighted 211% value compared to the DHEA changes of 406%). Similarly, following DHEA gel administration, the 249% increase in serum DHEA translates into only 65%, 91%, 96%, 56% and 30% increases in the AUC_{0-24h} values of serum 4-dione, DHEA-S, E₁-S, ADT-G and 3α-diol-G, respectively.

Since the high level of glucuronidation in the intestine and liver explains the high serum level of ADT-G and 3α-diol-G [31] following oral administration of DHEA, the relatively small increase in serum E₁-S (60%) compared to the 167% increase in serum DHEA after oral DHEA indicates a 36% relative efficacy of transformation into estrogens. As shown earlier [27–29], the present data indicate that DHEA administered to postmenopausal women is predominantly transformed into androgens rather than into estrogens.

Table 1
AUC_{0–24h} values measured on the 1st and 14th days of dosing as well as their ratio

Steroid	DHEA (ng h/ml)	5-Diol (ng h/ml)	4-Dione (ng h/ml)	Testosterone (ng h/ml)	E1 (pg h/ml)	E2 (pg h/ml)	DHEA-S (µg h/ml)	E1-S (pg h/ml)	ADT-G (ng h/ml)	3α-Diol-G (ng h/ml)
2× 50 mg capsules										
1st dosing	153 (19)	19.0 (20)	40.2 (39)	9.47 (31)	745 (30)	136 (20)	108 (20)	4.81 (39)	4112 (24)	259 (44)
14th dosing	144 (26)	20.4 (25)	43.6 (27)	9.72 (23)	910 (23)	165 (25)	95.0 (16)	7.44 (36)	5607 (28)	453 (41)
14th/1st	0.94	1.07	1.08	1.03	1.22	1.21	0.88	1.55	1.36	1.75
4 g 10% cream										
1st dosing	107 (33)	13.7 (31)	12.3 (43)	8.35 (16)	680 (48)	147 (50)	10.7 (45)	4.83 (58)	404 (62)	50.6 (93)
14th dosing	273 (36)	39.7 (31)	22.8 (33)	8.77 (16)	847 (22)	175 (27)	19.9 (34)	7.96 (39)	977 (66)	114 (104)
14th/1st	2.55	2.90	1.85	1.00	1.24	1.19	1.86	1.65	2.42	2.25
4 g 10% gel										
1st dosing	101 (49)	10.3 (55)	13.3 (45)	8.76 (11)	620 (31)	214 (137)	11.2 (35)	5.53 (84)	254 (30)	38.3 (86)
14th dosing	188 (30)	27.2 (32)	21.3 (51)	8.04 (22)	785 (40)	152 (24)	18.6 (34)	9.11 (106)	455 (23)	60.3 (85)
14th/1st	1.86	2.64	1.60	0.96	1.27	0.71	1.66	1.65	1.79	1.57

Values within parenthesis represent % coefficient of variation. DHEA was administered by the oral route (2× 50 mg capsules) or following application on the skin of 4 g of 10% DHEA cream or 4 g of 10% gel.

Table 2
Pretreatment and 14th day AUC_{0–24h} values of DHEA and its metabolites

Steroid	DHEA (ng h/ml)	5-Diol (ng h/ml)	4-Dione (ng h/ml)	Testosterone (ng h/ml)	E1 (pg h/ml)	E2 (pg h/ml)	DHEA-S (µg h/ml)	E1-S (pg h/ml)	ADT-G (ng h/ml)	3α-Diol-G (ng h/ml)
Basal (pretreatment)	53.9	8.56	12.9	8.72	717	135	9.76	4.64	292	46.6
(A) 2× 50 mg capsules										
14th day	144	20.4	43.6	9.72	910	165	95.0	7.44	5607	453
14th/basal	2.67	2.38	3.38	1.11	1.27	1.22	9.73	1.60	19.2	9.74
(B) 4 g 10% cream										
14th day	273	39.7	22.8	8.77	847	175	19.9	7.96	977	114
14th/basal	5.06	4.63	1.77	1.01	1.18	1.30	2.04	1.72	3.34	2.45
(C) 4 g 10% gel										
14th day	188	27.2	21.3	8.04	785	152	18.6	9.11	455	60.3
14th/basal	3.49	3.18	1.65	0.92	1.09	1.13	1.91	1.96	1.56	1.30

DHEA was administered by the oral route or percutaneously by cream or gel. Basal AUC_{0–24h} values were calculated by multiplying the pretreatment basal serum steroid levels including screening by 24 h.

7. Discussion

The present data clearly show that during chronic treatment with DHEA by cream or gel, the concentration of all the steroids rapidly reaches a plateau with no detectable change in the serum concentration of any of the steroids measured during daily application of DHEA on the skin. Accordingly, from 24 h after first administration of DHEA percutaneously, the concentration of all steroids remains at the same level, thus showing that daily application of DHEA on the skin maintains constant serum levels of DHEA and all its metabolites. In postmenopausal women, it is already known that the circadian variation of serum DHEA is relatively small compared to the situation in normally cycling premenopausal women [32].

The present data also show that following daily oral administration of DHEA, there is no significant accumulation of DHEA or of its metabolites. Moreover, the metabolism of DHEA following its administration by the oral or percutaneous route is quantitatively similar, the quantitative differences being explained by the entero-hepatic metabolism following oral administration.

The higher AUC_{0-24h} values of serum DHEA-S, ADT-G and 3α -diol-G combined with the lower AUC_{0-24h} values of DHEA and 5-diol following oral versus percutaneous administration indicate that metabolism through the gastrointestinal tract and/or first passage through the liver leads not only to a higher level of transformation of DHEA into DHEA-S through the activity of DHEA-sulfotransferase [33] but also to an increased metabolism of DHEA into androgens and their inactivation through the activity of liver glucuronosyltransferases [31,34,35]. In fact, as shown in Table 1, the exposure to DHEA of 144 ng h/ml (AUC_{0-24h}) on the 14th day of oral administration of 100 mg of DHEA leads to AUC_{0-24h} values of 5607 ng h/ml and 453 ng h/ml for ADT-G and 3α -diol-G, respectively. On the other hand, after percutaneous administration with the 10% DHEA cream, the AUC values for DHEA, ADT-G and 3α -diol-G are 273 ng h/ml, 977 ng h/ml and 114 ng h/ml, respectively. Thus, after oral administration, 1 ng h/ml of DHEA corresponds to an AUC value of 42.1 ng h/ml for the combination of the two metabolites of androgens (ADT-G + 3α -diol-G) while following application of the DHEA cream, 1 ng h/ml of DHEA exposure corresponds to 4.0 ng h/ml for the sum of the two androgen metabolites. Such data indicate that administration of DHEA by the oral route leads to an approximately 10-fold higher level of transformation of DHEA into ADT-G and 3α -diol-G than after percutaneous administration, at least at the doses used. When the same calculations are made for the data obtained after administration of DHEA by gel, an exposure to DHEA of 1 ng h/ml is accompanied by an AUC_{0-24h} value of 2.7 ng h/ml for ADT-G + 3α -diol-G, thus indicating an even higher ratio between oral and percutaneous DHEA administration.

As shown in Table 1, while a DHEA AUC_{0-24h} value of 1 ng h/ml leads to an AUC_{0-24h} value of 660 ng h/ml for

DHEA-S following oral administration of DHEA, corresponding values of 73 ng h/l and 99 ng h/l are observed after application of the precursor steroid by cream or gel. There is thus a 6.7–9.0-fold higher amount of DHEA-S in the circulation following the same exposure to circulating DHEA (serum AUC_{0-24h} value) after oral compared to percutaneous administration of DHEA under the conditions tested. The present data show a comparable influence of the passage of DHEA through the gastro-intestinal tract and the liver on serum DHEA-S, ADT-G and 3α -diol-G levels.

Although a lower difference is seen, relatively higher levels of serum 4-dione are observed after oral administration of DHEA compared to percutaneous administration of the precursor steroid. Thus, after oral administration of DHEA, a 1 ng h/ml value of the DHEA AUC_{0-24h} leads to a 0.3 ng h/ml 4-dione AUC_{0-24h} value while values of 0.08 ng h/ml and 0.11 ng h/ml are observed after administration of DHEA by cream and gel, respectively. As measured in the circulation, the transformation of DHEA into 4-dione is thus 2.70–3.76 times higher following oral compared to percutaneous administration of DHEA.

The data of Table 2 show that the DHEA AUC_{0-24h} value is increased by 167% over control following the daily oral administration of 100 mg DHEA compared to pretreatment basal levels while the daily percutaneous administration of 4 g of 10% DHEA cream and gel increases the serum DHEA levels by 406% and 249%, respectively. Since 400 mg of DHEA were applied on the skin compared to 100 mg by the oral route, and assuming linearity, the present data indicate that the oral route is 2.9- and 4.8-fold more efficient compared to the formulation used for the DHEA cream and gel, respectively.

In a study also performed in postmenopausal women, the oral administration of 150 mg and 300 mg of micronized DHEA resulted in maximal serum DHEA-S, DHEA and testosterone of approximately 1.5 mg/ml, 15 ng/ml and 2.75 ng/ml after the 300 mg dose and 10 μ g/ml, 12 μ g/ml and 1.6 ng/ml after the 150 mg DHEA dose, respectively [36]. Examination of these early results shows that a 20-fold increase in serum DHEA-S led to only a 6.9-fold increase in serum testosterone while serum DHEA was increased to 11.6-fold. Moreover, when the measured serum testosterone values are adjusted to one-third to take into account the two-thirds non-specific binding in the radioimmunoassay, the serum testosterone levels remained within the physiological levels during the 12 h which follow the administration of the 150 mg DHEA dose [36].

Similar differences observed between the oral and percutaneous routes for serum DHEA are seen for 5-diol which is transformed directly from DHEA by 17β -hydroxysteroid dehydrogenase [37]. In fact, while the 5-diol AUC_{0-24h} value is increased by approximately 138% over control after oral administration of 100 mg of DHEA, increases of 363% and 218% are measured after application of 400 mg of DHEA cream and gel, respectively.

As mentioned above, man is unique, with some other primates, in having adrenals that secrete large amounts of the

precursor steroids DHEA and DHEA-S, which are converted into 4-dione and then into potent androgens and/or estrogens in peripheral intracrine tissues [4,8–14]. It is thus remarkable that man, in addition to possessing very sophisticated endocrine and paracrine systems, has largely vested in sex steroid formation in peripheral tissues [5,14]. In fact, while the ovaries and testes are the exclusive sources of androgens and estrogens in lower mammals, the situation is very different in man and higher primates, where active sex steroids are in large part or wholly synthesized locally in peripheral tissues, thus providing target tissues with controls which adjust the formation and metabolism of sex steroids to local requirements.

Adrenal secretion of DHEA and 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 and 30 years. Thereafter, serum DHEA and DHEA-S levels decrease markedly [6]. In fact, at 70 years of age, serum DHEA-S levels are decreased to approximately 20% of their peak values, while they can decrease by 95% by the age of 85–90 years [6,16]. The 70–95% reduction in the formation of DHEA and DHEA-S by the adrenals during aging results in a dramatic reduction in the formation of androgens and estrogens in peripheral target tissues [28]. Such a marked decrease in the formation of sex steroids in peripheral tissues could well be involved in the pathogenesis of a series of diseases associated with aging.

As mentioned earlier, transformation of DHEA and DHEA-S into active androgens and/or estrogens in peripheral target tissues depends upon the level of expression of the various steroidogenic and metabolizing enzymes in each cell type [5]. Elucidation of the structure of most of the tissue-specific genes that encode the steroidogenic enzymes responsible for the transformation of DHEA and DHEA-S into androgens and/or estrogens has permitted rapid progress in this area [8–12,37–41].

The data showing the presence of relatively high levels of androgen metabolites in normal women [27–29] strongly suggest that the androgens play a major physiological but still underestimated role in women. The 44.5% fall which occurs in serum DHEA from 20 to 30 years of age to the age of 40–50 years in women [28] could well explain the bone loss which precedes menopause. In fact, age-related bone loss has been reported to begin in the fourth decade and changes in bone turnover have been found well before menopause [42–44]. In agreement with these findings, bone density was lower at all sites examined in women classified as perimenopausal compared to premenopausal [45]. In agreement with these findings, the changes in precursor androgen secretion by the adrenals precede by 10–20 years the decrease in ovarian estrogen secretion which abruptly stops at menopause [28].

It is important to realize that not only serum DHEA and DHEA-S decrease by 50% between the ages of 21 years and 50 years but that a similar decrease is observed for serum testosterone [46]. Such data could well suggest that hormone replacement therapy with androgens or their precursor(s)

should start early at menopause in order to compensate for this early fall in the secretion of androgen precursors by the adrenals and the parallel decrease in serum testosterone [47].

The active androgens and estrogens synthesized in peripheral target tissues exert their activity in the cells of origin and very little diffusion of the active sex steroids occurs, thus resulting in very low levels in the circulation. In fact, as observed previously [27] and confirmed in the present study, the most striking effects of DHEA administration are seen on the circulating levels of the glucuronide derivatives of the metabolites of DHT, namely ADT-G and 3 α -diol-G while no significant or only minor changes are seen in the serum levels of testosterone, E1 or E2. These active steroids are produced locally in the peripheral intracrine tissues which possess the appropriate steroidogenic enzymes to synthesize DHT from the adrenal precursors DHEA and DHEA-S as well as the enzymes that transform DHT into the inactive metabolites ADT and 3 α -diol which are further modified by glucuronidation [31].

In a recent study, daily oral administration of 50 mg of DHEA had no significant effect on serum testosterone or DHT while DHEA and ADT-G were increased to a similar extent (80–90%) [48]. In another study, predosing serum levels of DHEA-S in postmenopausal women were increased from 0.55 μ g/ml to about 1.4 μ g/ml [49], after daily oral administration of 25 mg of DHEA for 6 months. Serum DHEA and testosterone levels, however, measured 23 h after last administration of DHEA, were not changed significantly. Another study has indicated that the 50 mg daily oral dose of DHEA leads to serum androgen levels in the premenopausal range [36].

The present data clearly demonstrate that DHEA and DHEA-S are converted in specific peripheral intracrine tissues into active androgens and/or estrogens which can exert their biological effects at their site of synthesis with no or only small release of active steroids in the circulation. Accordingly, changes in serum levels of testosterone, E1 or E2 cannot be used as parameters of transformation of DHEA into androgens or estrogens [28]. In fact, the active steroids are metabolized in the same cells where they have been synthesized and exerted their action into inactive glucuronidated and sulfated metabolites which finally diffuse in the extracellular compartment and can be measured in the circulation [27–29]. Measurement of the conjugated metabolites of androgens is the only approach that permits an accurate estimate of the total androgen pool in women. It is most likely that a similar situation exists for estrogens, although a precise evaluation of the pharmacokinetics of estrogen metabolism and identification of their metabolites remains to be established.

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