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ScienceDirect
Journal of Steroid Biochemistry & Molecular Biology 103 (2007) 178–188
The Journal of Steroid Biochemistry & Molecular Biology
www.elsevier.com/locate/jsbmb

Metabolism of DHEA in postmenopausal women following percutaneous administration

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Received 13 July 2006; accepted 11 September 2006

Abstract

The marked decline in serum dehydroepiandrosterone (DHEA) with age is believed to play a role in health problems associated with aging, these health issues being potentially preventable or reversible by the exogenous administration of DHEA.

In the present study, liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) and gas chromatrography/mass spectrometry (GC/MS) were used to measure the serum levels of DHEA and 11 of its metabolites in seventy-five 60–65-year-old Caucasian women who received 3 g of 0.1%, 0.3%, 1.0% or 2.0% DHEA cream or placebo applied twice daily on the face, upper chest, arms and legs. The serum levels of DHEA increased 574% over control at the 2.0% DHEA dose while the sum of the androgen metabolites androsterone glucuronide (ADT-G), 3α-androstenediol-3G (3α-diol-3G) and 3α-diol-17G increased by only 231%. On the other hand, serum testosterone and dihydrosterone were increased by 192% and 275%, respectively, above basal levels compared to 139% and 158% for estrone and estradiol. Such data show that the transformation of exogenous DHEA in postmenopausal women is preferentially into androgens rather than into estrogens. On the other hand, the present data indicate that serum DHEA measurements following DHEA supplementation in postmenopausal women are an overestimate of the formation of active androgens and estrogens and suggest a decreased efficiency of transformation of DHEA into androgens and estrogens with aging.

Keywords: Menopause; Intracrinology; Sex steroids; Androgens; Estrogens; DHEA; Androsterone glucuronide; Androstanediol glucuronide; Percutaneous; Steroid assays; Mass spectrometry

1. Introduction

It is remarkable that man, in addition to possessing very sophisticated endocrine and paracrine systems for steroids has largely vested in sex steroid formation in peripheral tissues [1–7]. In fact, while the ovaries and testes are the exclusive sources of androgens and estrogens in lower mammals [8], the situation is very different in man and higher primates [9,10], where active sex steroids are in large part or wholly synthesized locally in peripheral tissues from the precursor dehydroepiandrosterone (DHEA), thus providing target tissues with the appropriate controls to adjust the formation and metabolism of sex steroids according to local requirements.

The marked reduction in the formation of DHEA by the adrenals during aging [11–15] results in a dramatic fall in the formation of androgens and estrogens in peripheral target tissues, a situation believed to be associated with age-related

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doi:10.1016/j.jsbmb.2006.09.034

health problems such as insulin resistance [16,17] and obesity [18–20]. On the other hand, much attention has been given to the benefits of DHEA administered to postmenopausal women, especially on the bone, skin, muscle, vaginum, adipose tissue, insulin resistance and well being after oral and percutaneous administration [4,21–23].

Following cessation of estrogen secretion by the ovaries in postmenopausal women, all estrogens and almost all androgens are made locally from DHEA in the peripheral target tissues with minimal diffusion of the active steroids outside these tissues. Consequently, when one desires to have access to markers of total sex steroid activity, it becomes essential to measure the steroid metabolites which represent the obligatory exit route of all steroids and are measurable in the circulation. 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 [24]. This recent completion of the identification and characterization of all the human UDP-glucuronosyl transferases makes possible the use of the glucuronide derivatives of androgens as markers of total androgenic activity in both women and men.

Since, as mentioned above, DHEA is believed to play an important role against various problems associated with menopause and aging, it was felt important to investigate in detail the metabolism of various doses of DHEA administered percutaneously. This route of administration was chosen in order to avoid the first passage of DHEA through the liver. Liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) and gas chromatography/mass spectrometry (GC/MS) were used to measure nine androgens, their precursors and metabolites as well as estrone (E1), 17β-estradiol (E2) and E1-sulfate (E1-S) in five groups of 15 postmenopausal women who received 3.0 g of 0.1%, 0.3%, 1.0% or 2.0% DHEA or placebo cream applied on the skin, twice daily.

2. Subjects

Seventy-five (75) healthy postmenopausal Caucasian women aged 60–65 years participated in this study after IRB approval and having given written informed consent. No subject had taken hormone replacement therapy during the previous 6 months. No subject was suffering from an endocrine disorder, and none was under treatment with lipid- or glucose-lowering agents. There was no active or history of thromboembolic disease, significant metabolic or endocrine disease and no clinically significant gastrointestinal, liver or gallbladder disease. There was no migraine and no diabetes mellitus not controlled by conventional therapy. There was no corticosteroid treatment within 6 weeks of study entry as well as treatment with β-carotenoid, retinoic acid, hydroquinone, α-hydroxyacid (including inhaled, topical or oral). There was no hypertension equal to or above 160/95 mmHg or not controlled by standard therapy as well as no confirmed clinically significant depression or confirmed severe psychiatric disturbance.

There was no administration of any investigational drug within 30 days of screening visit or previous treatment with androgens or anabolic steroids within 6 months prior to the screening visit. There was no exposure to or use of antidepressants, antipsychotics, or analgesics, within 30 days prior to enrollment. Smoking any number of cigarettes was an exclusion criteria. There was no former or present narcotic addiction or alcoholism. The body weight ranged between 18.5 and 29.9 of ideal body weight according to body mass index (BMI). There was no hepatic or renal impairment or condition known to affect drug or steroid metabolism. All subjects had a medical history, complete physical examination, serum biochemistry as well as complete blood and urinalysis.

Each subject was randomly assigned to one of the five treatment groups namely placebo, 0.1% DHEA, 0.3% DHEA, 1% DHEA and 2.0% DHEA. Daily, before breakfast, and after supper, for 13 weeks, subjects received 3.0 ml of one of the five emulsions. All subjects were instructed to apply the study treatment twice daily (in the morning between 6:00 and 9:30 h and in the evening between 18:00 and 21:30 h) during 13 weeks. The first application of the study treatment was carried out at the investigational site where instructions were provided to the subjects on how to apply the topical emulsion. Three hundred microliters (0.3 ml) of the emulsion were applied on the forehead including 2 cm past the hair line, 0.3 ml on the face (right side), 0.3 ml each on the back of right and left arms, 0.3 ml each on the external face of the right and left legs for a total dose of 3.0 ml of DHEA emulsion twice daily. For each woman, blood sampling was performed at the same time in the morning (±10 min) throughout the study, with the exception of the blood sample collected at screening visit, which was taken in the morning or afternoon up to 3 weeks prior to study initiation (day 1). On day 1 of the study, blood sampling was performed within 2 h prior to the first application of placebo or DHEA creams. The application of the cream was performed after blood sampling.

3. Materials and methods

3.1. Quantitation of steroids in human serum: human blood sample collection for measurement of DHEA and related steroids

The serum steroid levels of DHEA, DHEA-S, androst-5-ene-3β,17β-diol (5-diol), testosterone, androstenedione (4-dione), dihydrotestosterone (DHT), androsterone, estrone (E1), 17β-estradiol (E2), E1-sulfate (E1-S), androsterone glucuronide (ADT-G), androstane-3α,17β-diol-3-glucuronide (3α-diol-G) and 3α-diol-17G were measured by mass spectrometry at the Laboratory of Molecular

Endocrinology and Oncology, CHUL Research Center (Research Center of Le Centre Hospitalier de l'Université Laval).

3.2. Analysis of non-conjugated steroids

3.2.1. Preparation of calibration curve of standard samples and extraction of steroids from human serum

DHEA, 5-diol, 4-dione, testo, and DHT are analyzed by GC/MS. On each day of analysis, calibration standards ranging from 0.2 to 20 ng/ml for DHEA and 5-diol, from 0.05 to 10 ng/ml for 4-dione and testo, from 0.02 to 4 ng/ml for DHT, from 8.00 to 400 pg/ml for E1 and from 2.00 to 400 pg/ml for E2, are prepared using charcoal-adsorbed human serum.

For the extraction of steroids, 500 μl of a 0.5 M sodium acetate solution is added to each tube (1.0 ml for calibration standards). A methanolic solution (50 μl), containing a mixture of deuterated steroid internal standards, is then added to each tube. Aliquots of 0.75 ml of study samples (0.25 ml for calibration standards) are added and tubes are vortexed for ca. 1 min.

1-Chlorobutane (3 ml) is then added to each tube and mixed. After centrifugation, the organic extracts are collected and purified on LC-Si SPE columns. Columns and the adsorbed material are washed with ethyl acetate:hexane (ca. 6 ml; 1:9, v:v). The analytes of interest are then eluted using 4 ml ethyl acetate:hexane (50:50, v:v) which is evaporated at 50 °C. The dried residue is reconstituted in 0.5 ml ethyl acetate and vortexed for ca. 15 s.

An aliquot of 100 μl is transferred to a glass tube for the assay of 4-dione and the remaining extract is kept in the tube for the assay of DHEA, 5-diol, testo, DHT, E1 and E2. Both extracts are evaporated at 50 °C.

3.2.2. Derivatization of DHEA, 5-diol, testo, DHT, E1 and E2

Pentafluorobenzoylchloride in ethyl acetate (50 μl; 1/10, w/v) and pyridine in ethyl acetate (500 μl; 1:99, v:v) are added to the dried residue of DHEA, 5-diol, testo and DHT and the samples are incubated for ca. 30 min at 60 °C. After evaporation of the reagent mixture, a solution of 0.5 M $NaHCO_3$ (1.0 ml) is added to the tubes, which are then left to react for 15 min at room temperature. Hexane (2 ml) is then added to the tubes, which are vortexed for ca. 2 min and left at room temperature for ca. 10 min. The organic phase is evaporated at 50 °C and the final extract reconstituted in 50 μl isooctane and then transferred into a conical vial for injection into the GC/MS.

3.2.3. Derivatization of 4-dione

A solution of 1 mg pentafluorobenzylhydroxylamine/mL pyridine (100 μl) is added to the 4-dione extract and the tubes are incubated for ca. 30 min at 60 °C. After derivatization, the tubes are left to cool at room temperature for ca. 5 min and hexane (3 ml) is added to the samples. The mixtures are vortexed for ca. 5 s and then evaporated at 50 °C. The final extract is reconstituted in 50 μl isooctane and then transferred into a conical vial for injection into the GC/MS system.

3.2.4. Analysis by GC/MS

The GC/MS system for the analysis of DHEA, 5-diol, 4-dione, testo, DHT, E1 and E2 uses a 50% phenyl-methyl polysiloxane (DB-17HT) capillary column (30 m × 0.25 mm internal diameter, 0.15 μm film thickness) with helium as the carrier gas. The analytes and IS are detected using a HP5973 quadrupole mass spectrometer equipped with a chemical ionization source.

3.3. Analysis of the conjugated steroids—ADT-G, 3α-diol-3G and 3α-diol-17G

3.3.1. Preparation of calibration curve of standard samples and extraction from human serum

ADT-G, 3α-diol-3G and 3α-diol-17G are analyzed by a LC/MS/MS method using TurboIonSpray. On each day of analysis, calibration standards ranging from 2 to 200 ng/ml for ADT-G and from 0.50 to 50 ng/ml for 3α-diol-3G and 3α-diol-17G are prepared using a mixture of charcoal-adsorbed serum:water (1:1, v:v).

For extraction from serum, 500 μl of serum sample is transferred to each tube. Water (500 μl) is added and the tubes are then vortexed. A methanolic solution (100 μl) containing the deuterated steroid internal standard is then added to each tube. A solution of NaH_2PO_4/citric acid buffer (1.5 ml) is added and the tubes are vortexed again.

Samples are transferred to the C-18 SPE columns. Each column is then washed with water and a solution of methanol:water (50:50, v:v). The analytes of interest are then eluted using a solution (4 ml) of methanol:water (80:20, v:v), containing 10 mM ammonium acetate. The eluates are evaporated at 45 °C and the dried residue is reconstituted in a solution (100 μl) of methanol:water (50:50, v:v) containing 0.01% acetic acid prior to analysis.

3.3.2. Analysis by LC/MS/MS

The HPLC system uses a 150 mm × 4.6 mm, 4-μm particle size Synergy Hydro-RP column at a flow rate of 1.0 ml/min. ADT-G, 3α-diol-3G and 3α-diol-17G are detected using a Sciex API 3000 triple quadrupole mass spectrometer, equipped with TurboIonSpray™.

3.4. Analysis of the conjugated steroids DHEA-sulfate (DHEA-S) and E1-S

3.4.1. Preparation of calibration curve standard samples and extraction from human serum

DHEA-S and E1-S are analyzed by a LC/MS/MS method using TurboIonspray. On each day of analysis, calibration standards ranging from 0.075 to 10 μg/ml for DHEA-S and from 0.075 to 10 ng/ml for E1-S are prepared using PBS:charcoal adsorbed serum (1:1, v:v).

For extraction from serum, 100 μl of the serum sample is transferred to individual tubes and 2 ml of PBS buffer is added. A methanolic solution (50 μl) containing the deuterated steroid internal standard is then added to each tube. Samples are transferred on Oasis HLB SPE columns and each column is washed with water and methanol:water (10:90, v:v). The analytes of interest are then eluted with 4 ml of methanol. Methanol is evaporated at 35 °C and the dried residue reconstituted in 125 μl of methanol:water (50:50, v:v) and then filtered on 0.2 μm nylon filter. Part of this solution (20 μl) is diluted in 0.5 ml of methanol:water (50:50, v:v) containing 5 mM ammonium acetate and 0.01% acetic acid for the DHEA-S analysis. The remaining part is used directly for E1-S analysis.

3.4.2. Analysis by LC/MS/MS

For DHEA-S analysis, the HPLC system uses a 100 × 3.2-mm, 5-μm particle size, Phenomenex Columbus C18 column at a flow rate of 0.5 ml/min. DHEA-S is detected using a Sciex API 300 or API 3000 triple quadrupole mass spectrometer equipped with TurboIonspray™. For E1-S analysis, the HPLC system uses a 100 × 4.6 mm, 35 μm particle size Phenomenex Luna C18 column at a flow rate of 1.0 ml/min. E1-S is detected using a Sciex API 365 or API 3000 triple quadrupole mass spectrometer, equipped with Turbolonspray.

3.4.3. Coefficients of variation of steroid assays by mass spectrometry

The intra- and inter-assay coefficients of variation are indicated on Table 1.

3.4.4. Statistical analysis

The means and standard errors (S.E.M.) of serum concentrations were calculated for each group and timepoints (Figs. 2 and 3). Since the serum concentrations of most of steroids had reached a plateau at 1 week, the values obtained in each woman at 1 week and after were considered as replicates after treatment. A paired *t*-test was applied by pairing these replicates with the mean of two basal values (screening and day 1) for each subject. The *p* values were corrected by the Bonferroni formula to take account of the multiple statistical tests. The mean serum values and 5th to 95th centiles in 47 normal cycling premenopausal women 30–35 years old are provided to illustrate normal ranges observed in normal young women.

Table 1
Intra- and inter-assay coefficients of variation (%) for 12 steroids measured by GC/MS and LC–MS/MS in human samples (endogenous steroids)

Steroid	ng/ml	Coefficient of variation	
		Within runs	Between runs
DHEA[a]	2.44	2.0	1.9
DHEA-S[b]	593	5.2	6.3
5-Diol[a]	0.91	9.5	13.1
4-Dione[a]	0.62	3.7	4.2
Testo[a]	0.48	2.9	3.4
DHT[a]	0.11	3.1	4.1
ADT-G[b]	22.8	3.1	3.7
3α-Diol-3G[b]	0.79	10.3	10.7
3α-Diol-17G[b]	1.65	4.6	5.3
E1[a]	60.8	1.8	1.8
E2[a]	19.2	3.5	3.7
E1-S[b]	279	4.5	6.0

[a] GC/MS.
[b] LC–MS/MS.

4. Results

In order to better visualize the effect of DHEA administration on the serum levels of DHEA and 11 steroids derived from DHEA, Fig. 1 illustrates the pathways of transformation of DHEA into its multiple metabolites, including testosterone, DHT and the glucuronide derivatives of androgens which are the main parameters under investigation in the present study (Fig. 1).

As illustrated in Figs. 2 and 3, serum DHEA as well as all its metabolites have already reached maximal values 1 week following twice daily administration of 3 g of the DHEA cream at all doses. With 15 subjects in each group on day 1, there were 13–15 subjects per group at week 13 for the 0%, 0.1% and 0.3% DHEA creams while there were 10 and 3 subjects remaining for the 1.0% and 2.0% DHEA creams, respectively. In these two groups, 5 and 10 women, respectively, were stopped at 8 weeks for serum levels of DHEA above 11 ng/ml. Since the women having the highest serum DHEA values were those stopped at week 8, the values obtained at week 13 for the remaining women were not retained in the calculation in order to avoid bias.

Since the serum concentration of most steroids had reached a plateau at 1 week, the values obtained at 1, 2, 4, 8 and 13 weeks were pooled for the 0.1% and 0.3% creams while values obtained at 1, 2, 4 and 8 weeks were pooled for the 1.0% and 2.0% creams. These pooled values were compared to basal values as well as to the values found in 47 normal cycling premenopausal women of 30–35 years of age (Figs. 4 and 5). This analysis is important in order to compare the concentrations achieved following treatment of postmenopausal women with the various concentrations of the DHEA creams with the serum concentrations of the same steroids observed in normal young women.

From a baseline value measured at 1.80 ± 0.10 ng/ml, serum DHEA increased to 2.23 ± 0.17 ng/ml ($p < 0.005$), 4.07 ± 0.37 ng/ml ($p < 0.01$), 7.91 ± 0.41 ng/ml ($p < 0.01$), and 12.13 ± 1.28 ng/ml ($p < 0.01$) at the doses of 0.1%, 0.3%, 1.0% and 2.0% DHEA, respectively for an increase of 574% over control at the highest dose (Table 2). These values are to be compared with serum DHEA concentrations of 4.47 ± 0.32 ng/ml in 30–35 year-old premenopausal women with 5th and 95th centile values of 1.53 and 9.14 ng/ml, respectively (Fig. 4A). Serum DHEA-S, on the other hand, increased from 502 ± 28 to 1232 ±

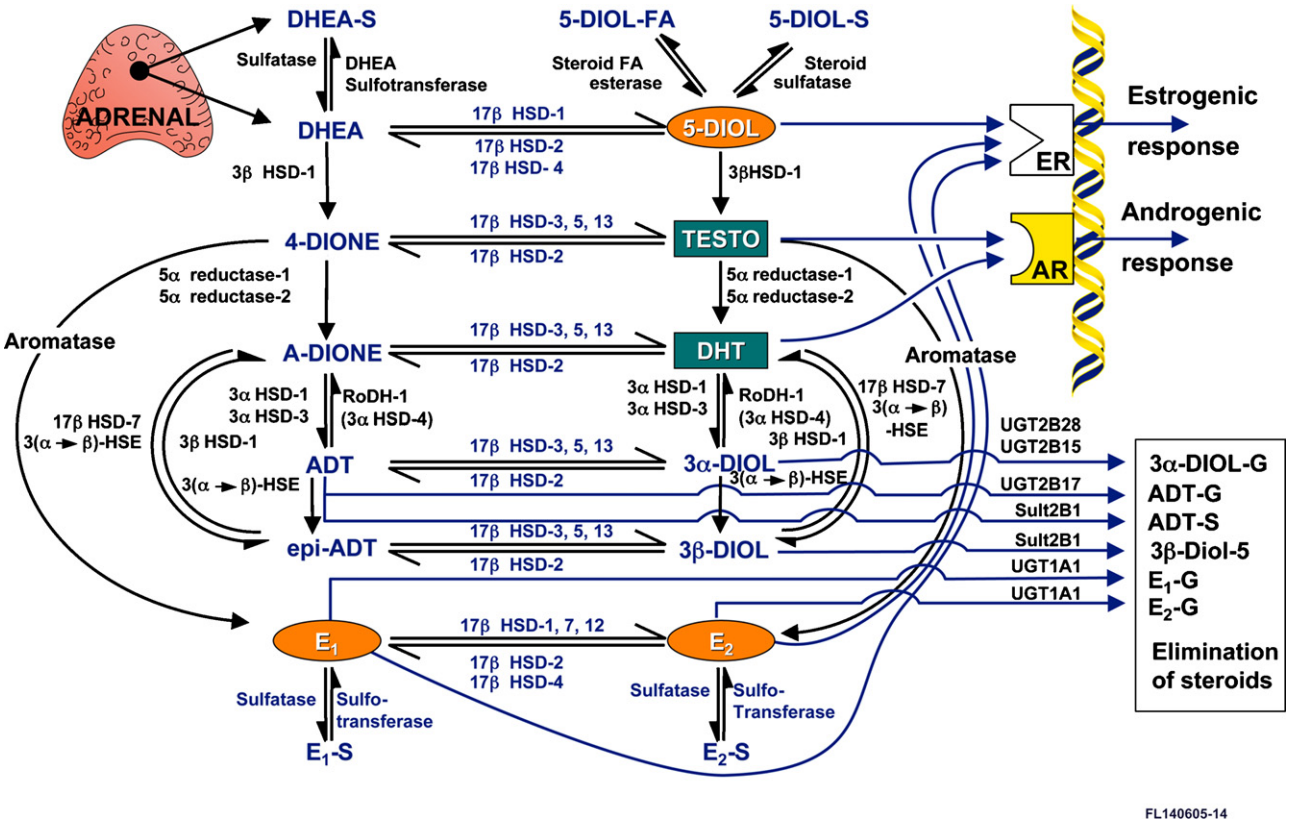

Fig. 1. Human steroidogenic and steroid-inactivating enzymes in peripheral intracrine tissues.

147 ng/ml at the highest dose (145% increase over control (Fig. 4B)).

As illustrated in Fig. 4C, serum 5-diol followed a pattern comparable to DHEA with a progressive increase from 0.22 ± 0.01 to 1.67 ± 0.16 ng/ml with the 2.0% DHEA cream (659% increase over control). Serum 4-dione, on the other hand, showed a progressive increase somewhat comparable to that of DHEA-S going from 0.36 ± 0.02 at baseline to 0.87 ± 0.05 ng/ml (142% increase over control) at the highest dose (Fig. 4D). The serum concentration of 4-dione remained well within the range of concentrations found in normal young women having an average of 0.96 ng ± 0.05 ng/ml and values of 0.45–1.64 ng/ml for the 5th and 95th centiles, respectively, a finding analogous to DHEA-S (Fig. 4B).

Testosterone, on the other hand, increased from a baseline of 0.13 ± 0.01 to 0.38 ± 0.04 ng/ml (192% increase over baseline) at the highest DHEA dose while the 5th to 95th centiles in young women are 0.06 and 0.31 ng/ml, respectively (Fig. 4E). Serum DHT follows a pattern comparable to that of testo with an increase from 0.04 ± 0.002 to 0.15 ± 0.02 ng/ml with the 2.0% DHEA cream, for a maximal 275% increase over baseline (Fig. 4F).

As observed for DHEA, 5-diol, 4-dione, testo and DHT, the three glucuronide derivatives accounting for all the elimination products of androgens were progressively increased with increasing doses (Fig. 5A–C). The maximal increase for the ADT-G, 3α-diol-3G and 3α-diol-17G are 223%, 295% and 402% over baseline, respectively (Table 2).

Serum estrone (E1), on the other hand, increased from a baseline of 17.58 ± 1.22 to 42.09 ± 6.96 pg/ml at the highest dose (139% over baseline) (Fig. 5D). Serum estradiol (E2) followed a comparable pattern with the first significant increase being seen at the 0.3% DHEA cream concentra-

Table 2
Baseline and 2.0% DHEA cream stimulated serum levels of DHEA and its 11 metabolites

Steroid	Baseline	2.0% DHEA cream	% increase over control
DHEA	1.80 ± 0.10[a]	12.13 ± 1.28	574
DHEA-S	502 ± 28[a]	1232 ± 147	145
5-Diol	0.22 ± 0.01[a]	1.67 ± 0.16	659
4-Dione	0.36 ± 0.02[a]	0.87 ± 0.05	142
Testo	0.13 ± 0.01[a]	0.38 ± 0.04	192
DHT	0.04 ± 0.002[a]	0.15 ± 0.02	275
ADT-G	13.60 ± 1.05[a]	43.90 ± 3.47	223
3α-Diol-3G	0.56 ± 0.05[a]	2.21 ± 0.21	295
3α-Diol-17G	0.47 ± 0.05[a]	2.36 ± 0.26	402
E1-sulfate	232 ± 23[b]	544 ± 110	134
17β-Estradiol	3.53 ± 0.30[b]	9.12 ± 1.20	158
E1	17.58 ± 1.22[b]	42.09 ± 6.96	139

[a] ng/ml.
[b] pg/ml.

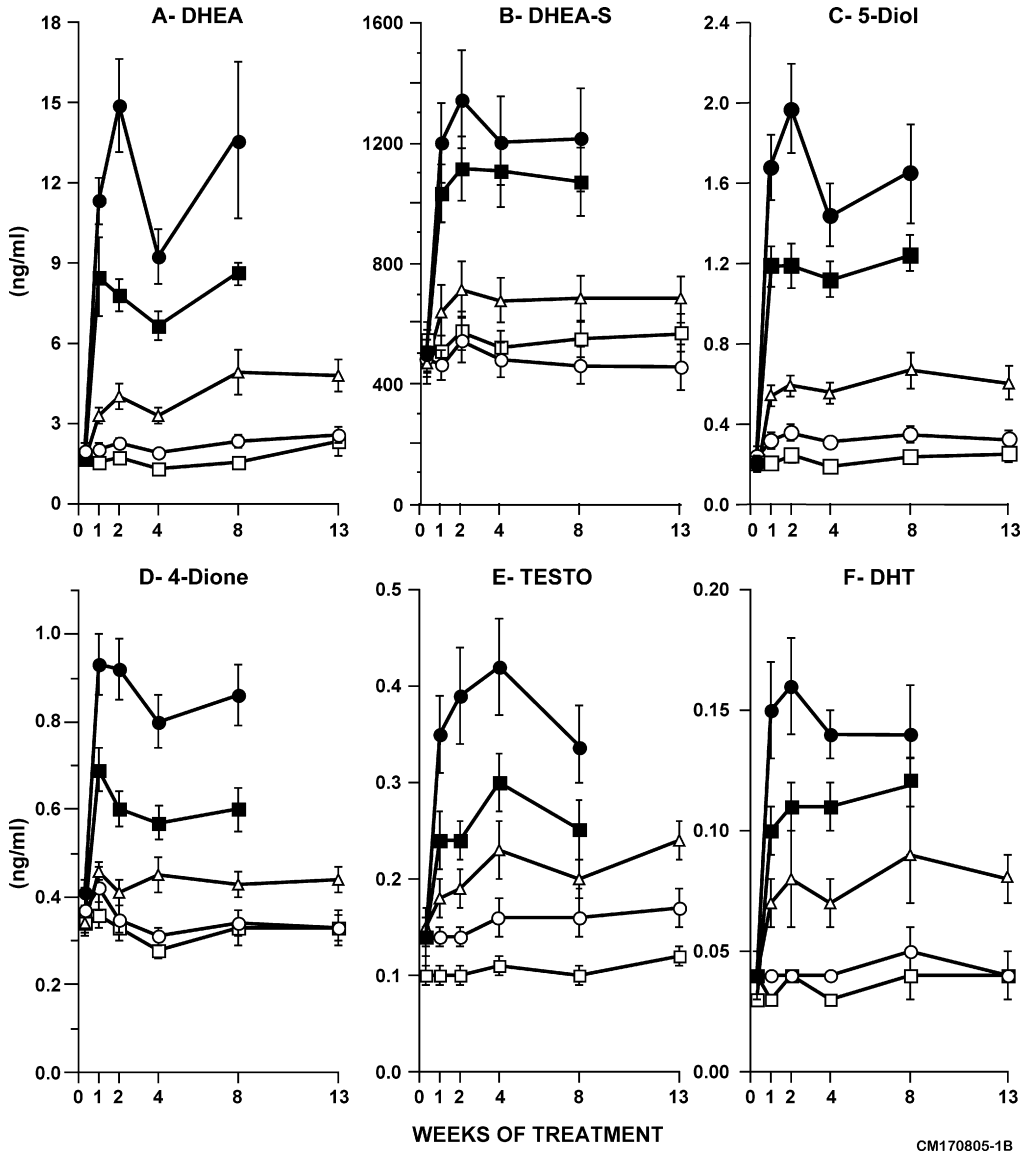

Fig. 2. Serum levels of (A) dehydroepiandrosterone (DHEA), (B) dehydroepiandrosterone sulfate (DHEA-S), (C) androst-5-ene-3β,17β-diol (5-DIOL), (D) androstenedione (4-DIONE), (E) testosterone (TESTO) and (F) dihydrotestosterone (DHT) in 55-65 year-old post-menopausal women following twice daily percutaneous application of placebo emulsion (□) or 0.1%(◯), 0.3% (△), 1.0% (■-) or 2.0% (●) DHEA emulsion for up to 13 weeks. Data are expressed as means ± S.E.M. ($n = 13$–15).

tion. The serum E2 levels increased from 3.53 ± 0.30 pg/ml (baseline) to 6.07 ± 0.82 and 9.12 ± 1.20 pg/ml at the 1.0% and 2.0% DHEA concentrations, respectively (Fig. 5E) for a 158% increase over control at the highest dose. It can be seen that serum E1-S follows a pattern almost identical to E1 and E2 with a maximal 134% increase over control from a baseline of 232 ± 23 pg/ml to 544 ± 110 pg/ml at the highest DHEA dose.

5. Discussion

The present study is the first one to measure by GC/MS and LC/MS/MS, the best available technology, the changes in serum steroid levels induced by the exogeneous administration of DHEA in the human. Since DHEA has been administered percutaneously, thus avoiding the high level of metabolism which accompanies the first pass through the liver at the higher and transient DHEA concentrations seen after oral administration, the present results should closely mimic the physiological metabolism of endogenous DHEA secreted by the adrenals.

A first interesting observation is that the 574% increase over basal levels of the serum DHEA concentration observed with the 2.0% DHEA cream is not accompanied by the same amplitude of changes in the serum levels of any other steroid (except 5-diol), including the glucuronide metabolites of androgens (Fig. 6, Table 2). Such data suggest

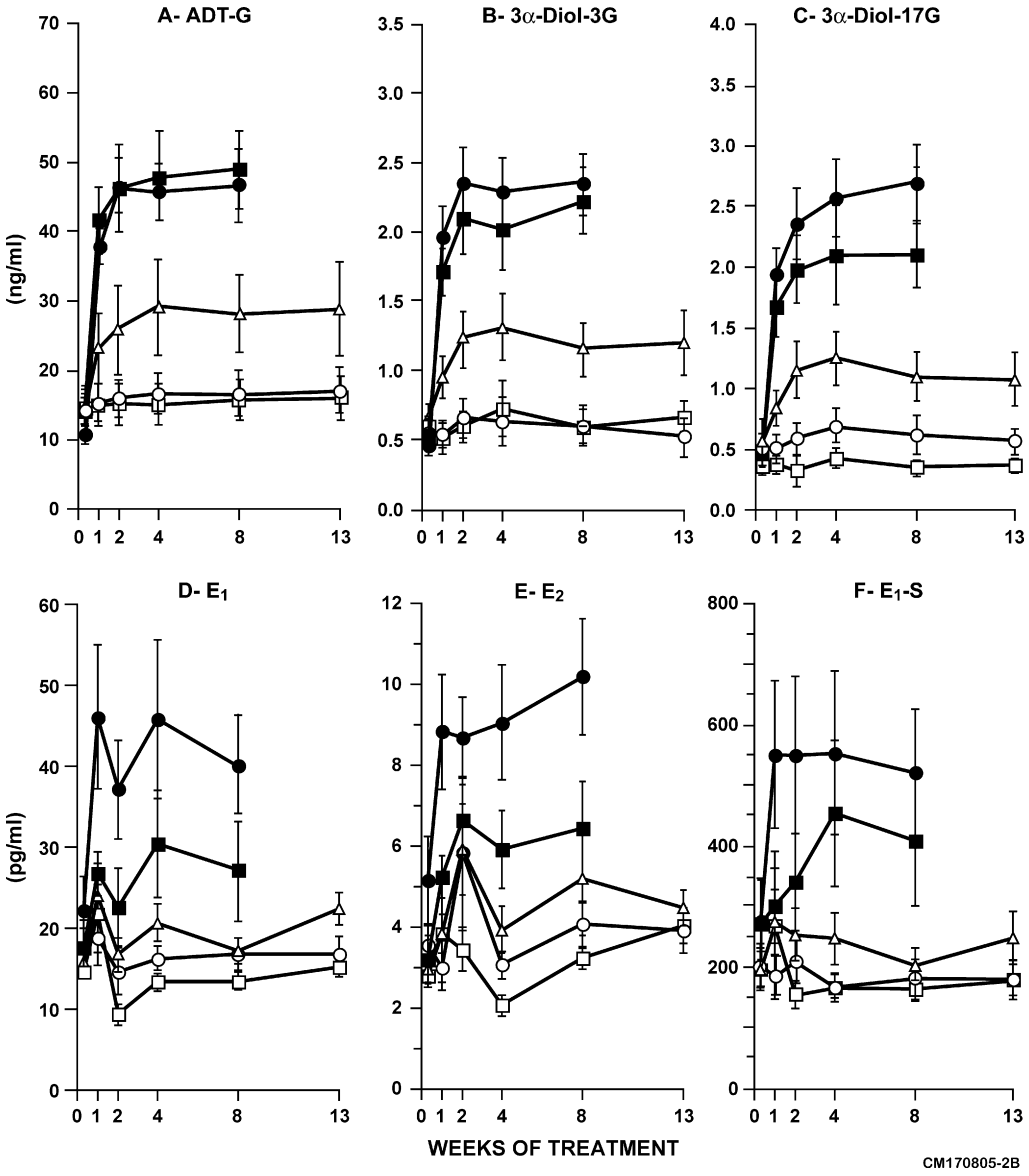

Fig. 3. Serum levels of (A) androsterone glucuronide (ADT-G), (B) androstane-3α,17β-diol 3-glucuronide (3α-DIOL-3G), (C) androstane-3α,17β-diol 17-glucuronide (3α-DIOL-17G), (D) estrone (E_1), (E) estradiol (E_2) and (F) estrone sulfate (E_1-S) in 55–65-year-old post-menopausal women following twice daily percutaneous application of placebo emulsion (□) or 0.1%(◯), 0.3% (△), 1.0% (■) or 2.0% (●) DHEA emulsion for 13 weeks. Data are expressed as means ± S.E.M. (n = 13–15).

that, at least in postmenopausal women, the serum DHEA concentrations measured after DHEA administration are a significant overestimate of the effect of DHEA on the circulating levels of the active androgens (Table 2). In fact, the 574% increase over control of serum DHEA leads to only 192% and 275% increases of serum testo and DHT, respectively. More significantly, since all androgens are eliminated from the organism as the glucuronide derivatives of ADT and 3α-diol that must necessarily pass through the circulation, the 574% increase in serum DHEA leads to only a 231% increase in the sum of the serum levels of ADT-G, 3α-diol-3G and 3α-diol-17G (Table 2), thus indicating that a 574% increase in serum DHEA translates only to a 231% increase in the total formation of androgens. Such data indicate that the efficacy of transformation of exogenous 2.0% DHEA cream into androgens is only 40% compared to the efficacy of the enzymatic machinery under basal conditions (Figs. 6 and 7). Taking the efficacy of transformation of DHEA under basal conditions as 100%, the efficacy observed with the 0.3% and 1.0% creams decreases to 79% and 72%, respectively, to reach, as mentioned above, only 40% with the 2.0% DHEA cream (Figs. 6 and 7).

The apparent saturation of the enzymatic process is best illustrated in Fig. 6 showing a complete saturation of the formation of androgen metabolites at 7.9 ng DHEA/ml. This

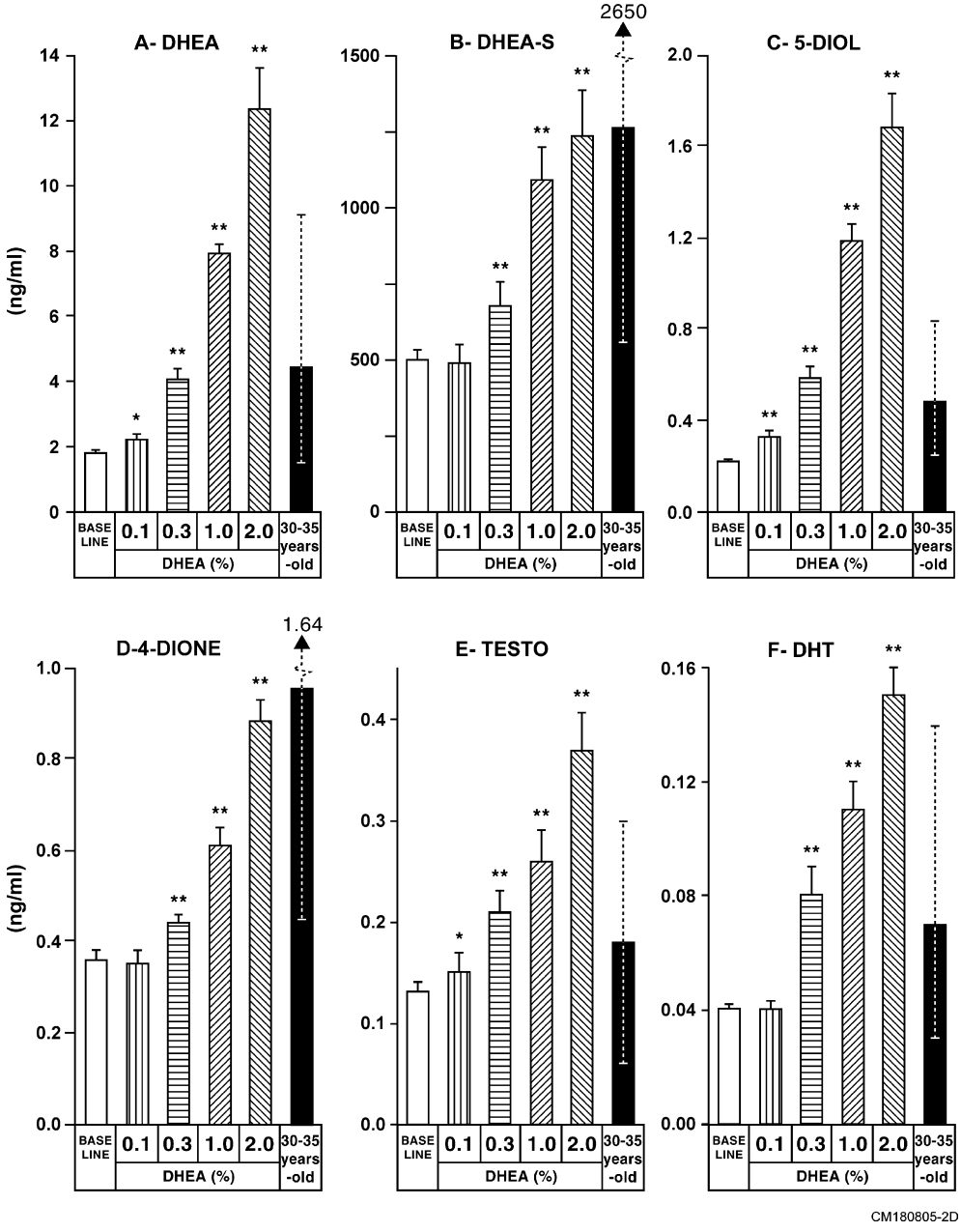

Fig. 4. Serum levels of (A) DHEA, (B) DHEA-S, (C) 5-DIOL, (D) 4-DIONE, (E) TESTO and (F) DHT measured up to week 13 following percutaneous administration of 0.1%, 0.3%, 1.0% or 2.0% DHEA emulsion in 55–65-year-old post-menopausal women. Data are expressed as means ± S.E.M. Serum steroid levels measured in 30–35-year-old premenopausal women are added as reference. Data are expressed as mean ($n = 47$) while the 5th and 95th centiles are indicated (dashed lines). $^{*}p < 0.05$, $^{**}p < 0.01$, experimental vs. baseline values.

figure shows that this saturation value of serum DHEA is close to but still below the 95th centile of the serum DHEA concentration observed in control postmenopausal women, namely 9.1 ng/ml. Such data suggest that the administration of DHEA to postmenopausal women can lead to high serum levels of the steroid precursor DHEA which is not automatically transformed into active androgens due to a limiting factor in the enzymatic machinery leading to androgen formation.

It is also of interest to see in Table 2 that the transformation of exogenous DHEA into estrogens is even inferior to the transformation into androgens. Thus, at the highest DHEA concentration used, serum E1 and E2 increased by 139% and 158% over baseline, respectively, while serum testosterone and DHT increased by 192% and 275%, respectively. Serum E1-S, on the other hand, increased only 134% over control under the same conditions while, the sum of the androgen metabolites increased by 231%. It is thus quite clear from

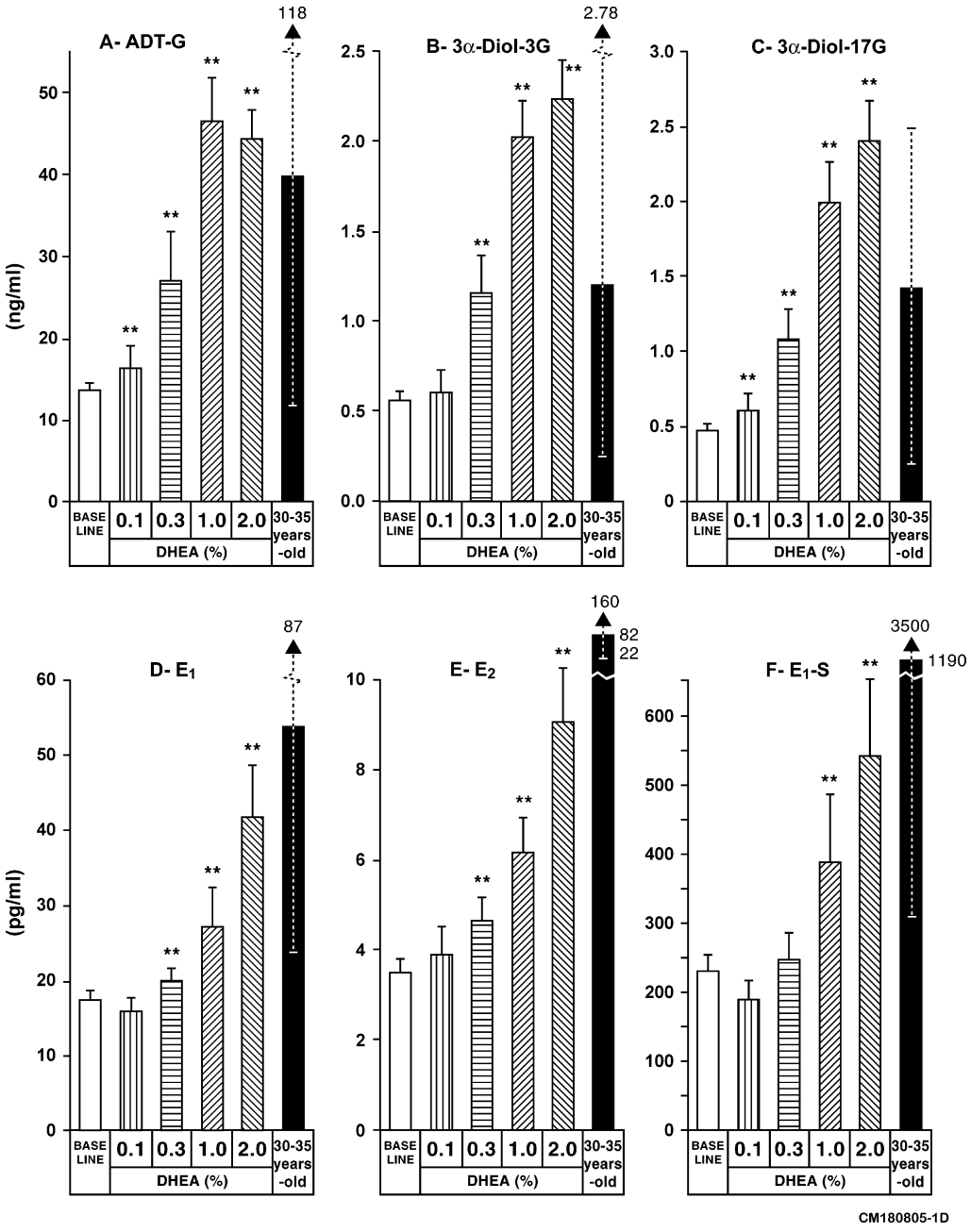

Fig. 5. Serum levels of (A) ADT-G, (B) 3α-DIOL-3G, (C) 3α-DIOL-17G, (D) E_1, (E) E_2 and (F) E_1-S measured up to week 13 following percutaneous administration of 0.1%, 0.3%, 1.0% or 2.0% DHEA emulsion in 55–65-year-old post-menopausal women. Data are expressed as means ± S.E.M. Serum steroid levels measured in 30–35 year-old premenopausal women are added as reference. Data are expressed as mean ($n = 47$) while the 5th and 95th centiles are indicated (dashed lines). $^{*}p < 0.05$, $^{**}p < 0.01$, experimental versus baseline values.

the present data that DHEA is preferentially transformed into androgens rather than into estrogens in postmenopausal women.

It should be mentioned that in 30–35-year-old premenopausal women, the average serum DHEA concentration of 4.47 ng/ml is accompanied by total serum levels of ADT-G, 3α-diol-3G and 3α-diol-17G of 42.85 ± 15.68 with values of 12.7 ng/ml and 123.6 ng/ml for the 5th and 95th centiles, respectively (unpublished data). Thus, under basal conditions, DHEA is transformed into the glucuronide derivatives of androgens with a similar efficacy in both premenopausal 30–35-year-old women (unpublished data) and in 60–65 year-old postmenopausal women of the present study. In fact, dividing the sum of serum ADT-G, 3α-diol-3G and 3α-diol-17G by the serum DHEA concentration gives a value of 9.59 for premenopausal women (42.85–4.47 ng/ml) while 8.13 is observed for postmenopausal subjects (14.63–1.80 ng/ml). It is to be remembered that the serum concentrations of ADT-

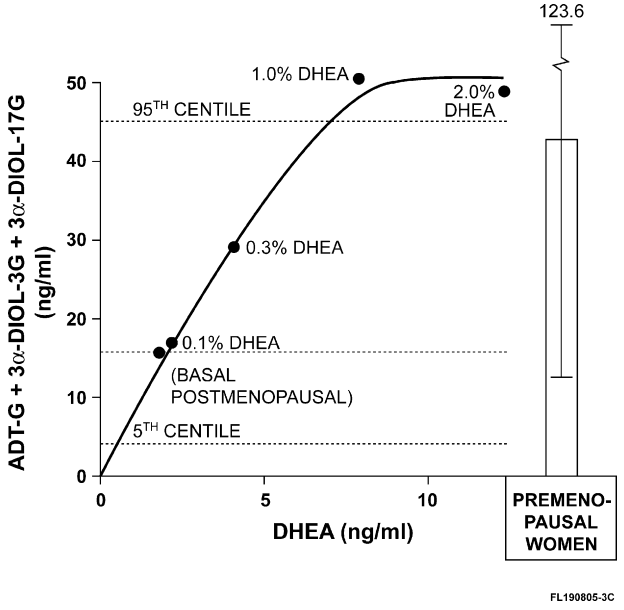

Fig. 6. Effect of increasing serum concentrations of DHEA induced by the twice daily percutaneous administration of 3 g of 0% (placebo), 0.1%, 0.3%, 1.0% or 2.0% DHEA cream on the sum of the serum levels of the androgen metabolites ADT-G, 3α-diol-3G and 3α-diol-17G expressed in ng/ml.

G, 3α-diol-3G and 3α-diol-17G represent the sum of all the androgen metabolites released from all tissues, thus leaving the possibility that this apparent decrease in the capacity to metabolize DHEA with age could well not apply to all tissues.

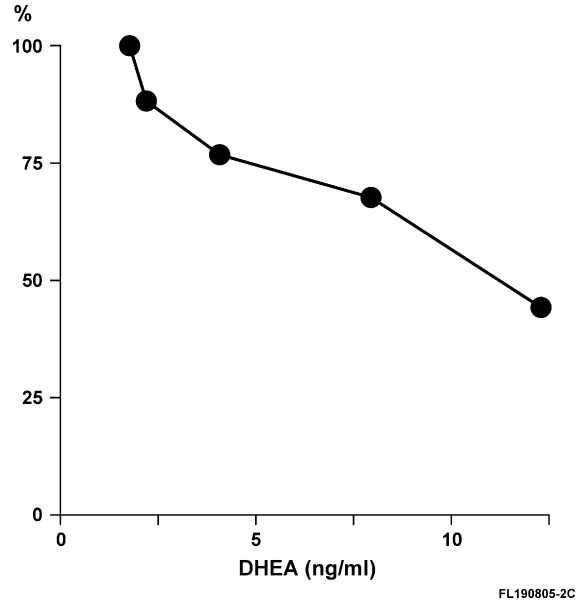

Fig. 7. Efficacy of transformation of DHEA into androgen metabolites (ADT-G + 3α-diol-3G + 3α-diol-17G) in postmenopausal women. The data are expressed as percentage of the transformation observed under basal serum DHEA concentrations of 1.80 ± 0.10 ng/ml taken as 100%. The efficacy is obtained by dividing the sum of the serum levels of ADT-G, 3α-diol-3G and 3α-diol-17G by the serum DHEA levels.

Acknowledgements

We thank Micheline Harvey for her skilful typing and preparation of the manuscript.

This study was sponsored by Endorecherche and l'Oréal.

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