

# Androgen glucuronides, instead of testosterone, as the new markers of androgenic activity in women

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## Abstract

Despite the long series of cohort studies performed during the last 20 years, the correlation between serum testosterone and any clinical situation believed to be under androgen control in women has remained elusive. This is likely related to the recent finding that the androgens made locally in large amounts in peripheral tissues from the precursor dehydroepiandrosterone (DHEA) act in the same cells where synthesis takes place and are not released in significant amounts in the circulation, thus making unreliable the measurement of serum testosterone as marker of total androgenic activity. The objective is to determine if serum androgen glucuronides can be replaced by testosterone or another steroid as measure of androgenic activity.

Since the glucuronide derivatives of androgens are the obligatory route of elimination of all androgens, these metabolites were measured by liquid chromatography tandem mass spectrometry under basal conditions in 377 healthy postmenopausal women aged 55–65 years as well as in 47 premenopausal women aged 30–35 years while testosterone was assayed by gas chromatography mass spectrometry. No correlation was found between the serum concentration of testosterone and that of androsterone glucuronide (ADT-G) or androstenediol glucuronide (3 $\alpha$ -diol-G), the androgen metabolites which account for the total pool of androgens.

The present data show that measurement of the total pool of androgens reflected by the serum levels of ADT-G and 3 $\alpha$ -diol-G cannot be replaced by serum testosterone or any other steroid, including DHEA or DHEA sulphate. These findings may have implications for women with androgen deficiency involving osteoporosis, obesity, type 2 diabetes, sexual dysfunction, loss of muscular strength and a series of other clinical situations affecting women's health. Measuring ADT-G and 3 $\alpha$ -diol-G might identify cases of true androgen deficiency and provide an opportunity to offer appropriate androgen therapy.

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**Keywords:** Testosterone; Androsterone; Glucuronides; Intracrinology; Mass spectrometry; Androgenic activity marker

## 1. Introduction

It has been a long-lasting problem to realize, report after report, that the long series of case-control and prospective

cohort studies which analyzed the correlation between serum testosterone and the incidence of obesity, insulin resistance, breast cancer, sexual dysfunction or other clinical problems in women always yielded contradictory results (reviewed in Refs. [1–10]). Such a lack of correlation is difficult to reconcile with the well-demonstrated stimulation of sexual function [4,7,9] and the inhibitory effects of exogenous androgens on obesity and breast cancer [1,3,11–13]. This lack of consistency between the serum levels of testosterone and the effect of exogenous androgens has raised serious

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doubts about the validity of measurements of total as well as free serum testosterone as markers of androgenic activity in women.

One explanation for the lack of correlation between serum testosterone and clinical parameters known to be under androgen control could be related to the recent data showing that the majority of androgens in women are made locally in peripheral target tissues from the inactive precursor dehydroepiandrosterone (DHEA) of adrenal gland origin [1,14,15]. Since the androgens made locally do not originate from circulating testosterone, one could reasonably expect that measurement of the serum levels of testosterone is of questionable biological and clinical significance. In fact, the androgens testosterone and dihydrotestosterone (DHT) made in peripheral tissues from DHEA of adrenal origin exert their action locally in the same cells where synthesis takes place with only minimal release as active androgens in the circulation. Following their formation and availability for local intracellular action, testosterone and DHT are inactivated and transformed in the same cells into water soluble glucuronide derivatives which diffuse quantitatively into the general circulation where they can all be measured before their elimination by the kidneys (Fig. 1). This recently identified mechanism of steroid formation and action has been named intracrinology [14,16].

In addition to the above-identified major issue about the biological significance of the serum testosterone concentration which does not take into account the large amount of androgens made in peripheral tissues, it should be mentioned that the radioimmunoassays generally used to measure serum sex steroids have questionable specificity [17–20].

Considering the major importance for the clinicians and women to have access to a valid and precise marker of andro-

genic activity in order to assess with confidence the role of androgens in a series of problems particularly frequent after menopause, namely type 2 diabetes, obesity and arteriosclerosis (metabolic syndrome), sexual dysfunction, osteoporosis, breast cancer, skin atrophy as well as loss of muscular strength, physical fitness and well-being, we have used liquid chromatography–tandem mass spectrometry (LC–MS/MS) and gas chromatography mass spectrometry (GC–MS) to measure nine androgens, their precursors and metabolites in 377 postmenopausal women in good health aged 55–65 years in order to analyze the correlation between serum testosterone and the true markers of the total pool of androgens, namely the glucuronide derivatives of androsterone (ADT) and androstane-3 $\alpha$ , 17 $\beta$ -diol (3 $\alpha$ -diol), the obligatory route of elimination of androgens. We have also compared with data obtained in 47 normal 30–35-year-old normally cycling women.

## 2. Subjects

Three hundred and seventy seven (377) healthy postmenopausal women aged 55–65 years and 47 premenopausal 30- to 35-year-old women 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. Subjects did not have active or history of thromboembolic disease, significant metabolic or endocrine disease or clinically significant gastrointestinal or liver or gallbladder disease. There was no migraine and no diabetes mellitus not controlled by conventional therapy. Subjects had no corticosteroid treatment within 6 weeks of study entry as well as treatment with  $\beta$ -carotenoid, retinoic acid, hydroquinone,  $\alpha$ -hydroxyacid (including inhaled, topical, oral). Hypertension equal to or above 160/95 mm Hg or not controlled by standard therapy as well as confirmed clinically significant depression or confirmed severe psychiatric disturbance were not permitted.

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 was permitted. The subjects had no exposure to or use of antidepressants, antipsychotics, narcotic and 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). No hepatic or renal impairment or condition known to affect drug or steroid metabolism was permitted. All subjects had a medical history, complete physical examination, serum biochemistry as well as complete blood and urinalysis. Blood sampling was performed under fasting conditions between 08:00 and 10:30 h.

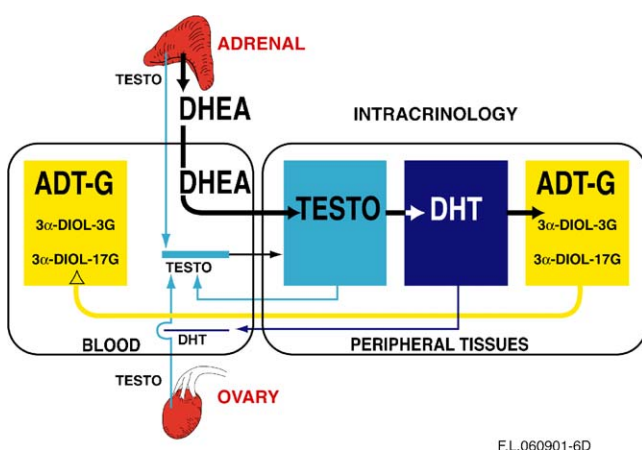


Fig. 1. Schematic representation of the very important contribution of the precursor DHEA of adrenal origin to total androgenic activity in postmenopausal women with a parallel minor contribution of testosterone of two origins, namely the ovary and adrenal. A very small proportion of the testosterone and DHT made intracellularly by the steroidogenic enzymes of the intracrine pathway diffuse into the circulation. The height of the bars is proportional to the concentration of each steroid.

### 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-3 $\beta$ , 17 $\beta$ -diol (5-diol), dihydrotestosterone (DHT), testosterone, androstenedione (4-dione), androsterone glucuronide (ADT-G), androstane-3 $\alpha$ , 17 $\beta$ -diol-3-glucuronide (3 $\alpha$ -diol-G) and 3 $\alpha$ -diol-17G were measured at the Laboratory of Molecular Endocrinology, CHUL Research Center.

#### 3.2. Analysis of non-conjugated steroids

##### 3.2.1. Preparation of calibration curve of standard samples

DHEA, 5-diol, 4-dione, testo, and DHT were 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 and from 0.02 to 4 ng/mL for DHT were prepared using charcoal-adsorbed human serum.

##### 3.2.2. Extraction of steroids from human serum

Briefly, 500  $\mu$ L of a 0.5 M sodium acetate solution is added to each tube (1.0 mL for calibration standards). A methanolic solution (50  $\mu$ L), containing a mixture of deuterated steroid internal standards, was then added to each tube. Aliquots of 0.75 mL of study samples (0.25 mL for calibration standards) were added and tubes were vortexed for ca. 1 min.

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

An aliquot of 100  $\mu$ L was transferred to a glass tube for the assay of 4-dione and the remaining extract was kept in the tube for the assay of DHEA, 5-diol, testo and DHT. Both extracts were evaporated at 50 °C.

##### 3.2.3. Derivatization of DHEA, 5-diol, testo and DHT

Pentafluorobenzoylchloride in ethyl acetate (50  $\mu$ L; 1/10; w/v) and pyridine in ethyl acetate (500  $\mu$ L; 1:99; v:v) were added to the dried residue of DHEA, 5-diol, testo and DHT and the samples were incubated for ca. 30 min at 60 °C. After evaporation of the reagent mixture, a solution of 0.5 M NaHCO<sub>3</sub> (1.0 mL) was added to the tubes, which were then left to react for 15 min at room temperature. Hexane (2 mL) was then added to the tubes which were vortexed for ca. 2 min and left at room temperature for ca. 10 min. The organic phase was evaporated at 50 °C and the final extract reconstituted in

50  $\mu$ L isooctane and then transferred into a conical vial for injection into the GC/MS.

##### 3.2.4. Derivatization of 4-dione

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

##### 3.2.5. Analysis by GC/MS

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

#### 3.3. Analysis of the conjugated steroids—ADT-G, 3 $\alpha$ -diol-3G and 3 $\alpha$ -diol-17G: preparation of calibration curve of standard samples

ADT-G, 3 $\alpha$ -diol-3G and 3 $\alpha$ -diol-17G were 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 3 $\alpha$ -diol-3G and 3 $\alpha$ -diol-17G were prepared using a mixture of charcoal-adsorbed serum:water (1:1;v:v).

##### 3.3.1. Extraction from human serum

Briefly, 500  $\mu$ L of serum sample were transferred to each tube. Water (500  $\mu$ L) was added and the tubes were then vortexed. A methanolic solution (100  $\mu$ L) containing the deuterated steroid internal standard was then added to each tube. A solution of NaH<sub>2</sub>PO<sub>4</sub>/citric acid buffer (1.5 mL) was added and the tubes were vortexed again.

Samples were transferred to the C-18 SPE columns. Each column was then washed with water and a solution of methanol:water (50:50;v:v). The analytes of interest were then eluted using a solution (4 mL) of methanol:water (80:20;v:v), containing 10 mM ammonium acetate. The eluates were evaporated at 45 °C and the dried residue was reconstituted in a solution (100  $\mu$ L) 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  $\times$  4.6 mm, 4  $\mu$ m particle size Synergy Hydro-RP column at a flow rate of 1.0 mL/min. ADT-G, 3 $\alpha$ -diol-3G and 3 $\alpha$ -diol-17G are detected using

a Sciex API 3000 triple quadrupole mass spectrometer, equipped with TurboIonSpray™.

3.4. Analysis of the conjugated steroid DHEA-sulfate (DHEA-S): preparation of calibration curve standard samples

DHEA-S was analyzed by a LC/MS/MS method using TurboIonSpray. On each day of analysis, calibration standards ranging from 0.075 to 10 µg/mL were prepared using PBS:charcoal adsorbed serum (1:1;v:v).

3.4.1. Extraction from human serum

Briefly, 100 µL of the serum sample was transferred to individual tubes and 2 mL of PBS buffer was added. A methanolic solution (50 µL) containing the deuterated steroid internal standard was then added to each tube. Samples were transferred on Oasis HLB SPE columns and each column was washed with water and methanol:water (10:90;v:v). The analytes of interest were then eluted with 4 mL of methanol. Methanol was 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) was 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.

3.4.2. Analysis by LC–MS/MS

For DHEA-S analysis, the HPLC system uses a 100 mm × 3.2 mm, 5 µm particle size, Phenomenex Columbus C18 column at a flow rate of 0.5 mL/min. DHEA-S was detected using a Sciex API 300 or 3000 triple quadrupole mass spectrometer, respectively, equipped with TurboIonSpray™. The coefficients of variation of the steroid assays by mass spectrometry are indicated in Table 1.

3.5. Statistical analysis

The distribution characteristics of the serum levels is presented in Table 1 for all measured androgen metabolites. Some values are graphically presented on Figs. 2–5. For the

Table 1  
Intra- and inter-assay coefficients of variation (%) for nine steroids measured by GC–MS<sup>X</sup> and LC–MS/MS<sup>XX</sup> in human samples (endogenous steroids)

Steroid	ng/ml	Coefficient variation	
		Within runs	Between runs
DHEA <sup>X</sup>	2.44	2.0	1.9
DHEA-S <sup>XX</sup>	593	5.2	6.3
5-diol <sup>X</sup>	0.91	9.5	13.1
4-dione <sup>X</sup>	0.62	3.7	4.2
Testo <sup>X</sup>	0.48	2.9	3.4
DHT <sup>X</sup>	0.11	3.1	4.1
ADT-G <sup>XX</sup>	22.8	3.1	3.7
3α-diol-3G <sup>XX</sup>	0.79	10.3	10.7
3α-diol-17G <sup>XX</sup>	1.65	4.6	5.3

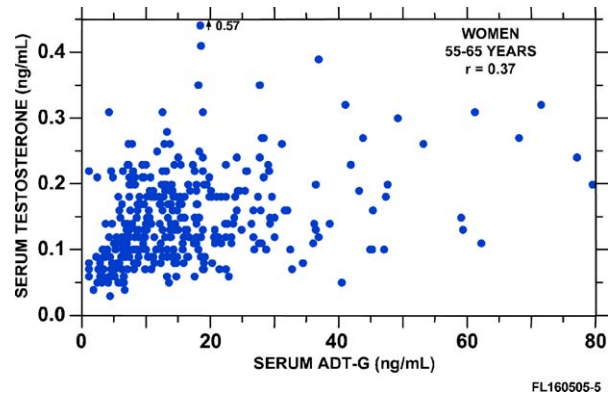


Fig. 2. Lack of correlation between serum ADT-G (androsterone glucuronide) and testosterone concentrations in three hundred seventy-seven (377) 55–65-year-old postmenopausal women. The Spearman correlation coefficient value of 0.37 is indicated.

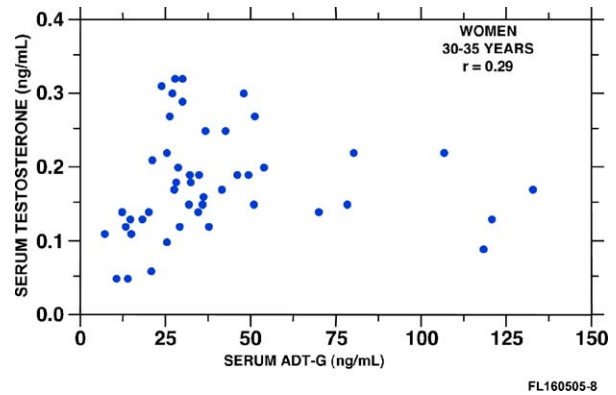


Fig. 3. Lack of correlation between serum testosterone and ADT-G levels in forty-seven (47) 30–35-year-old premenopausal women. The Spearman correlation coefficient value of 0.29 is indicated.

women aged 55–65 years, the distributions of DHEA, 4-dione and testo were fitted to lognormal distributions. However, for 5-diol, DHT, 3α-diol-3G, 3α-diol-17G, DHEA-S and ADT-G, a normal kernel density estimate was used to represent the distribution. For the women aged 30–35 years, distributions

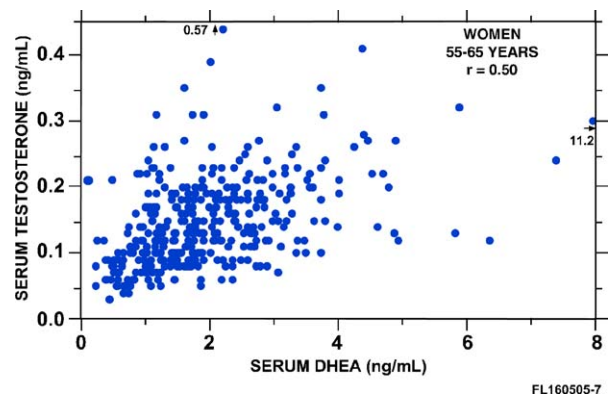


Fig. 4. Lack of correlation between serum testosterone and DHEA levels in three hundred seventy-seven (377) 55–65-year-old postmenopausal women. The Spearman correlation coefficient value of 0.50 is indicated.

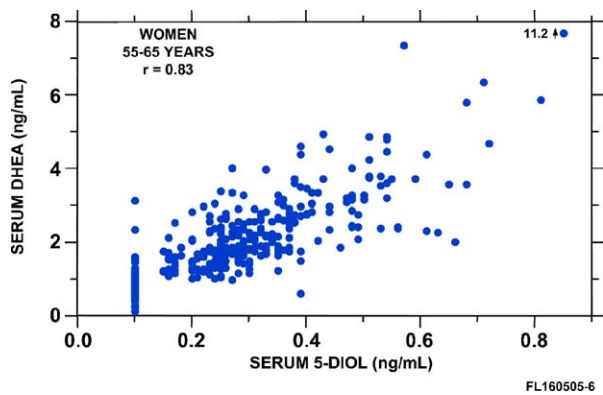


Fig. 5. Correlation between serum DHEA (dehydroepiandrosterone) and 5-diol (androst-5-ene-3 $\beta$ , 17 $\beta$ -diol) levels in three hundred seventy seven (377) 55–65-year-old postmenopausal women. The Spearman correlation coefficient value ( $r$ ) of 0.83 is indicated.

were fitted to lognormal distributions except for 3 $\alpha$ -diol-17G where a normal kernel density estimate was used. The closeness of the relationship between any two circulating androgens was estimated by the Spearman correlation coefficient (Table 2).

#### 4. Results

The serum levels of androgens as well as their precursors and glucuronide derivatives measured by mass spectrometry in 377 healthy postmenopausal women aged 55–65 years are shown in Table 2. For comparison, the values found in normal cycling 30–35-year-old women are shown in the same table.

As can be seen in Fig. 2 and Table 3, no useful correlation is found between serum testosterone and ADT-G ( $r=0.37$ ), this metabolite accounting by itself for 93% of the obligatory metabolites of androgen elimination. An even lower correlation is observed between serum testosterone and the serum levels of the two other androgen glucuronides, namely 3 $\alpha$ -diol-3G (androstane-3 $\alpha$ , 17 $\beta$ -diol-3G) ( $r=0.27$ ) and 3 $\alpha$ -diol-17G ( $r=0.22$ ) (Table 3). A similar lack of correlation between testosterone and ADT-G ( $r=0.29$ ) is seen in normal cycling 30- to 35-year-old women (Fig. 3). A somewhat better but still poor correlation is observed between serum testosterone and DHEA, the main source of androgens in women, with a  $r$ -value of 0.50 (Fig. 4) or between DHEA and ADT-G with a  $r$ -value of 0.65 (Table 3).

In fact, testosterone is, among all the steroids measured, the one showing the lowest correlation with the three glucuronide derivatives of androgens. Such data suggest that variable rates of secretion of testosterone by the ovary and/or adrenal could be responsible for the lack of correlation of ADT-G and 3 $\alpha$ -diol-G with serum testosterone which is the sum of testosterone of ovarian and adrenal origins secreted directly into the blood plus the testosterone diffusing from the peripheral tissues following peripheral transformation of DHEA into androgens (Fig. 1). It is also possible that the

Table 2  
Serum steroid levels in post-menopausal women aged 55–65 years as well as in normal cycling women aged 30–35 years

Status	DHEA (ng/mL)	5-Diol (ng/mL)	4-Dione (ng/mL)	Testo (ng/mL)	DHT (ng/mL)	ADT-G (ng/mL)	3G (ng/mL) <sup>a</sup>	17G (ng/mL) <sup>b</sup>	DHEA-S (ng/mL)
55-65-year-old postmenopausal women ( $n=377$ )	Mean 1.95 S.D. 1.18 Median 1.72 5th–95th centiles (min.–max.) 0.56–3.99 (0.1–11.19)	0.27 0.15 0.25 0.1–0.54 (0.1–0.85)	0.40 0.18 0.37 0.17–0.71 (0.1–1.37)	0.14 0.070 0.13 0.06–0.26 (0.03–0.57)	0.04 0.03 0.03 0.01–0.07 (0.01–0.29)	15.83 12.46 13.11 3.27–41.72 (1.0–79.4)	0.64 0.52 0.55 0.25–1.69 (0.25–3.48)	0.57 0.47 0.25 0.25–1.54 (0.25–3.56)	0.59 0.36 0.55 0.15–1.24 (0.04–2.44)
30–35-year-old premenopausal women ( $n=47$ )	Mean 4.47 S.D. 2.19 Median 4.14 5th–95th centiles (min.–max.) 1.53–9.14 (1.41–10.37)	0.49 0.20 0.44 0.25–0.84 (0.25–0.96)	0.96 0.35 0.92 0.45–1.64 (0.31–1.77)	0.18 0.07 0.17 0.06–0.31 (0.05–0.32)	0.07 0.03 0.07 0.03–0.14 (0.03–0.17)	40.21 29.31 31.62 12.17–118.2 (6.86–132.6)	1.21 0.83 1.06 0.25–2.78 (0.25–4.33)	1.43 0.93 1.35 0.25–2.56 (0.25–5.71)	1.27 0.62 1.04 0.56–2.65 (0.45–2.71)

<sup>a</sup> Androstane-3 $\alpha$ ,17 $\beta$ -diol-3G.

<sup>b</sup> Androstane-3 $\alpha$ ,17 $\beta$ -diol-17G.

Table 3

Spearman correlation coefficients ( $r$ ) for serum steroid levels in postmenopausal women aged 55–65 years as well as in normal cycling women aged 30–35 years

Status	Steroid								
	DHEA	5-Diol	4-Dione	Testo	DHT	DHEA-S	ADT-G	3G	17G
55–65-year-old postmenopausal women ( $n = 377$ )									
DHEA	1.00	0.83	0.79	0.50	0.65	0.77	0.65	0.52	0.47
5-Diol	0.83	1.00	0.61	0.53	0.70	0.77	0.57	0.50	0.46
4-Dione	0.79	0.61	1.00	0.56	0.49	0.52	0.51	0.43	0.39
Testo	0.50	0.53	0.56	1.00	0.56	0.41	0.37	0.27	0.22
DHT	0.65	0.70	0.49	0.56	1.00	0.65	0.59	0.46	0.42
DHEA-S	0.77	0.77	0.52	0.41	0.65	1.00	0.62	0.55	0.45
ADT-G	0.65	0.57	0.51	0.37	0.59	0.62	1.00	0.71	0.59
3G	0.52	0.50	0.43	0.27	0.46	0.55	0.71	1.00	0.63
17G	0.47	0.46	0.39	0.22	0.42	0.45	0.59	0.63	1.00
30–35-year-old premenopausal women ( $n = 47$ )									
DHEA	1.00	0.89	0.70	0.46	0.43	0.61	0.55	0.55	0.44
5-Diol	0.89	1.00	0.65	0.62	0.60	0.66	0.46	0.46	0.39
4-Dione	0.70	0.65	1.00	0.73	0.47	0.54	0.44	0.55	0.60
Testo	0.46	0.62	0.73	1.00	0.65	0.44	0.29	0.38	0.45
DHT	0.43	0.60	0.47	0.65	1.00	0.43	0.44	0.35	0.39
DHEA-S	0.61	0.66	0.54	0.44	0.43	1.00	0.47	0.50	0.46
ADT-G	0.55	0.46	0.44	0.29	0.44	0.47	1.00	0.80	0.70
3G	0.55	0.46	0.55	0.38	0.35	0.50	0.80	1.00	0.73
17G	0.44	0.39	0.60	0.45	0.39	0.46	0.70	0.73	1.00

peripheral tissue-made testosterone that diffuses at a low level into the circulation could be highly variable and explain, at least partially, the lack of correlation with serum androgen glucuronides. Better correlations are observed, however, between serum DHEA and its  $17\alpha$ -reduced metabolite androstenediol (5-diol) ( $r = 0.83$ ) (Fig. 5), DHEA and androstenedione ( $r = 0.79$ ) as well as between DHEA and its sulphated metabolite DHEA-S ( $r = 0.77$ ) (Table 2).

## 5. Discussion

While one would ideally like to know the level of androgenic activity in each specific tissue, such a direct measurement of the intratissular concentration of the active androgens is not possible in the human except under exceptional circumstances such as in samples of tissue obtained at surgery [21–23]. However, while not permitting the assessment of androgenic activity in specific tissues, measurement of the glucuronide derivatives of ADT and  $3\alpha$ -diol by validated mass spectrometry techniques permits a precise measure of the total pool of androgens in the whole organism. In fact, it is now well established that uridine glucuronosyl transferase 2 B7 (UGT 2 B7), UGT 2 B15 and UGT 2 B17 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 now makes possible the use of the glucuronide derivatives of androgens as markers of the total pool of androgens in both women and men. ADT-S is also a metabolite excreted in large amounts but this steroid is exclusively of adrenal origin

and does not reflect androgenic activity in peripheral tissues [25].

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 [14–16,22,23]. The level of transformation of the adrenal precursor steroid DHEA into androgens and/or estrogens in peripheral target tissues thus depends upon the level of expression of the various steroidogenic enzymes in each cell of each tissue [1,14]. This situation of a high secretion rate of adrenal precursor sex steroids by the adrenals in men and women is thus completely different from all animal models used in the laboratory, namely rats, mice, guinea pigs, and all others (except monkeys), where the secretion of sex steroids takes place exclusively in the gonads and the adrenals do not secrete significant amounts of DHEA [23].

The classical concept of androgen and estrogen secretion in women assumed that all sex steroids had to be transported by the general circulation following secretion by the ovaries before reaching the target tissues. According to this classical concept, it was erroneously believed that the active steroids could be measured directly in the blood, thus providing a potentially easily accessible measure of the general exposure to sex steroids. In fact, this concept is valid only for the animal species lower than primates but it does not apply to the human, especially in postmenopausal women where all estrogens and almost all androgens are made locally from DHEA in the peripheral tissues which possess the enzymes required to synthesize the physiologically required active sex steroids. Such a local biosynthesis and action of androgens in target tissues eliminates the exposure of other tissues to androgens and thus minimizes the risks of undesirable masculinizing or

other androgen-related side effects [23]. The same applies to estrogens, although a reliable parameter of total estrogen secretion (comparable to the glucuronides identified for androgens) has yet to be determined. Although a fraction of androgens are aromatized to estrogens, the lack of sufficient information on the identity of the metabolites does not permit to make a sufficiently complete analysis of their metabolism at this time.

The present data show that the most practical and probably only valid means of assessing androgenic activity in women is to measure ADT-G, the metabolite that accounts for 93% of the total androgen glucuronide derivatives, by a validated liquid chromatography tandem mass spectrometry (LC–MS/MS) technique, thus replacing measurement of serum testosterone. This strategy should identify the cases of true androgen deficiency, thus offering the possibility to prescribe an appropriate androgen therapy or prevention regimen.

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