

Marked Decline in Serum Concentrations of Adrenal C19 Sex Steroid Precursors and Conjugated Androgen Metabolites During Aging

FERNAND LABRIE, ALAIN BÉLANGER, LIONEL CUSAN, JOSÉ-LUIS GOMEZ, AND BERNARD CANDAS

Laboratory of Molecular Endocrinology, CHUL Research Center, Le Centre Hospitalier Universitaire de Québec, and Laval University, Québec, G1V 4G2, Canada

ABSTRACT

The present data show a dramatic decline in the circulating levels of dehydroepiandrosterone (DHEA), DHEA-sulfate (DHEA-S), androst-5-ene-3 β ,17 β -diol (5-diol), 5-diol-sulfate, 5-diol-fatty acid esters, and androstenedione in both men and women between the ages of 20–80 yr. In the 50- to 60-yr-old group, serum DHEA decreased by 74% and 70% from its peak values in 20- to 30-yr-old men and women, respectively. The serum concentrations of the conjugated metabolites of dihydrotestosterone (DHT), namely androsterone (ADT)-G, androstane-3 α ,17 β -diol (3 α -diol-G), androstane-3 β ,17 β -diol (3 β -diol-G), and ADT-sulfate are the most reliable parameters of the total androgen pool in both men and women, whereas serum testosterone and DHT can be used as markers of testicular secretion in men and interstitial ovarian secretion in women. The serum concentration of these various conjugated androgen metabolites decreased by 40.8% to

72.8% between the 20- to 30-yr-old and 70- to 80-yr-old age groups in men and women, respectively, thus suggesting a parallel decrease in the total androgen pool with age. As estimated by measurement of the circulating levels of these conjugated metabolites of DHT, it is noteworthy that women produce approximately 66% of the total androgens found in men. In women, most of these androgens originate from the transformation of DHEA and DHEA-S into testosterone and DHT in peripheral intracrine tissues, whereas in men the testes and DHEA and DHEA-S provide approximately equal amounts of androgens at the age of 50–60 yr. An additional potentially highly significant observation is that the majority of the marked decline in circulating adrenal C₁₉ steroids and their resulting androgen metabolites takes place between the age groups of 20- to 30-yr olds and 50- to 60-yr-olds, with smaller changes are observed after the age of 60 yr. (*J Clin Endocrinol Metab* 82: 2396–2402, 1997)

THE MARKED decline with aging in the concentration of the circulating steroids of adrenal origin, especially dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S) is well recognized (1–3). However, only a limited number of adrenal and gonadal steroids have been measured during advancing age, especially in women, in which the impact of androgens and estrogens of adrenal origin is of particular importance (4). In fact, the metabolites of C₁₉-steroids, including sulfates, glucuronides, and fatty acid ester derivatives have received little attention in women, although it becomes clear that detailed knowledge about these metabolites is essential for a valid assessment of androgen and estrogen formation and action in peripheral intracrine tissues (5).

It is remarkable that humans, in addition to possessing very sophisticated endocrine and paracrine systems, have largely vested sex steroid formation in peripheral tissues. In fact, although the ovaries and testes are the exclusive sources of androgens and estrogens in lower mammals (6), the situation is very different in higher primates, in which active sex steroids are in a large part or whole synthesized locally in peripheral tissues, thus providing individual target tissues with the means to adjust formation and metabolism of sex steroids to their local requirements (4, 5, 7, 8). Thus, humans

and some other primates are unique in having adrenals that secrete large amounts of the precursor steroids DHEA-S and DHEA, which are converted into androstenedione (4-dione) and then into active androgens and/or estrogens in peripheral tissues (4, 7–9).

Transformation of the adrenal precursor steroids DHEA-S and DHEA into active androgens and/or estrogens in peripheral target tissues depends on the level of expression and activity of the various steroidogenic and metabolizing enzymes in each of these tissues. This new field of hormone formation and action has been called intracrinology (4, 8). Knowledge in this area has recently made rapid progress with the elucidation of the structure of most of the tissue-specific complementary DNAs and genes that encode the steroidogenic enzymes responsible for the transformation of DHEA-S and DHEA into androgens and/or estrogens in peripheral tissues (5, 10–14). The particular importance of DHEA and DHEA-S is best illustrated by the finding that approximately 50% of total androgens in adult men derive from these adrenal precursor steroids (7, 15, 16). In women, our best estimate of the intracrine formation of active estrogens in peripheral tissues from adrenal precursor steroids is approximately 75% before menopause and close to 100% after menopause (4).

To gain a better knowledge of the role of DHEA and DHEA-S transformation in both men and women, we analyzed the serum levels of 18 conjugated C₂₁- and C₁₉-steroids. We wanted to precisely assess the changes occurring in the serum concentration of these steroids over the range

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Address all correspondence and requests for reprints to: Fernand Labrie, Laboratory of Molecular Endocrinology, CHUL Research Center, 2705 Laurier Boulevard, Québec (Québec), G1V 4G2, Canada.

of ages from the peak value of adrenal secretion of DHEA and DHEA-S at the ages of 20–30 yr to the nearly lowest values found at the ages of 70–80 yr.

Subjects and Methods

Sixty healthy men and women (10 of each sex/age range of 20–30 yr, 30.1–40 yr, 40.1–50 yr, 50.1–60 yr, 60.1–70 yr, and 70.1–80 yr) participated in this study after IRB approval and having given their written informed consent. The participants were nonsmokers. No subject had taken hormone replacement therapy during the previous year. No subject was suffering from an endocrine disorder, and none was under treatment with lipid- or glucose-lowering agents. All subjects had a medical history, complete physical examination, serum biochemistry profile including lipids, complete blood count, urinalysis, and detailed serum hormone determinations during the screening phase of the protocol. Although there was no specific requirement for exercise and diet, no subject was involved in a weight loss program nor was following a special diet. Blood sampling was performed under fasting conditions between 0800–0900 h.

Steroid analysis in serum

Steroid extraction. Ethanol (5 mL) was added to 1 mL serum, and centrifugation was performed at $2000 \times g$ for 15 min. The resulting pellet was further extracted with 2 mL ethanol and, after a second centrifugation at $2000 \times g$ for 15 min, the two supernatants were combined. Pellets were then resuspended once again in 5 mL hexane to maximize the recovery of nonpolar steroids. The suspension was recentrifuged as described above, and the supernatant was decanted and combined with the two previously obtained ethanol extracts. The organic solvent was then evaporated under nitrogen, and the residue was dissolved in 1 mL water/methanol (95:5, vol/vol). The C-18 columns (Bound-Elut, Amersham, Bucks, UK) were conditioned by passing consecutively 10 mL methanol, 10 mL water, and 10 mL methanol/water (5:95, vol/vol). The extracts solubilized in water/methanol (95:5, vol/vol) were then deposited on the C-18 columns. After washing the columns with 2 mL water: methanol (95:5, vol/vol), 5 mL methanol/water (50:50, vol/vol) were added to eluate DHEA-S, after which 5 mL methanol/water (85:15, vol/vol) were added to eluate the nonconjugated steroids. The acylated steroids were then collected following the addition of 5 mL methanol.

Chromatography on LH-20 columns and RIA. Chromatography on Sephadex LH-20 columns (Pharmacia, Uppsala, Sweden) was performed as previously described (17). In brief, the nonconjugated steroids from the three fractions were solubilized in 1 mL isooctane/toluene/methanol (90:5:5, vol/vol/vol) and deposited on the LH-20 columns. The appropriate fractions were collected and, after evaporation of the organic solvent, the concentration of the various steroids was determined by RIA as previously described (17–19).

Calculations and statistic analyses

RIA data were analyzed using a program based on model II of Rodbard and Lewald (20). Plasma steroid levels are shown as the means \pm SEM of duplicate determinations of individual samples. Statistical significance was measured according to the multiple range test of Duncan-Kramer (21).

Results

From values of 22.7 ± 1.7 nM and 23.8 ± 3.4 nM at the age of 20–30 yr, serum DHEA decreased markedly to reach 4.5 ± 0.4 nM and 7.2 ± 1.5 nM at the age of 70–80 yr in men and women, respectively (Fig. 1A). This marked decrease in serum DHEA corresponds to 80.1% and 69.7% declines between the ages of 20–30 and 70–80 yr in men and women, respectively. At the ages of 40–50 yr, serum DHEA had already decreased by 40.9% ($P < 0.01$) and 44.5% ($P < 0.01$) in men and women, respectively. In analogy, at the ages of 50–60 yr serum DHEA was decreased by 74.4% and 70.5%

in men and women, respectively, compared with the peak values measured at the ages of 20–30 yr. It can be seen in Fig. 1 that the majority of the decline in serum DHEA occurred up to the age of 50–60 yr in both men and women, and that relatively smaller changes were observed up to the last time interval studied, 70–80 yr.

The serum DHEA-S concentration also declines dramatically with age in both men and women (Fig. 1B). From values of 11.5 ± 1.0 μ M measured in the youngest age group of men, serum DHEA-S decreased to 2.3 ± 0.3 μ M at the ages of 70–80 yr, whereas in women serum DHEA-S decreased from 6.2 ± 0.5 μ M to 1.6 ± 0.3 μ M, thus representing 80% and 74% declines, respectively.

As illustrated in Fig. 1C, the concentration of serum DHEA-fatty acid esters decreased from 9.2 ± 0.6 nM in 20- to 30-yr-old men to 1.9 ± 0.3 nM in the 70- to 80-yr-old group age (79.3% decline). In women, on the other hand, serum DHEA-fatty acid esters decreased from 9.3 ± 0.9 nM in the 20- to 30-yr-old group to 4.1 ± 0.5 nM (56% decline) in the 60- to 70-yr-old group and to 3.8 ± 0.5 nM (59% decrease) in the 70- to 80-yr-old group of women.

Aging had similar effects on serum androst-5-ene-3 β ,17 β -diol (5-diol), the steroid formed by 17 β -hydroxysteroid dehydrogenase (17 β -HSD) from DHEA and the immediate precursor of the androgen testosterone obtained through 3 β -hydroxysteroid dehydrogenase (3 β -HSD) activity (Fig. 1D). Serum 5-diol decreased from 5.1 ± 0.2 nM in 20- to 30-yr-old men to a value of 2.0 ± 0.2 nM in 60- to 70-yr-old men (60.7% decrease). This was followed by a nonsignificant slight increase at 2.5 ± 0.2 nM measured in the 70- to 80-yr-old group. In women, serum 5-diol decreased from 3.0 ± 0.2 nM in the youngest age group to 1.5 ± 0.1 nM in 70- to 80-yr-old subjects (50% decrease).

It was next of interest to see the changes associated with aging on the serum concentration of 4-dione, the immediate product of DHEA formed by 3 β -HSD (22). 4-Dione is the precursor of the androgen testosterone and the estrogen estrone (E_1) through the action of 17 β -HSD and aromatase, respectively. As can be seen in Fig. 2A, serum 4-dione decreased in men from 3.5 ± 0.3 nM in the 20- to 30-yr-old group to 1.7 ± 0.2 nM in the oldest age group (51.4% decline). In contrast, in women serum 4-dione decreased from 3.7 ± 0.3 nM to 1.4 ± 0.2 nM at the age of 50–60 yr (62% decrease), with a slight but not significant increase at the later ages.

In contrast, no significant or consistent change was measured in serum testosterone levels in either men and women during aging (Fig. 2B). Serum dihydrotestosterone (DHT) showed a decrease in men at the ages of 50–70 yr, whereas in women a progressive decrease from 0.9 ± 0.1 nM to 0.5 ± 0.05 nM (44% decrease) was seen between the age ranges of 20–30 and 70–80 yr (Fig. 2C).

Because the serum concentrations of testosterone and DHT in men are 90–95% related to the secretion of androgens by the testes (7, 16), these measurements do not reflect the total pool of androgens, especially the important contribution of the adrenals in the total androgen pool. It is thus of particular interest to measure the metabolites of DHT, namely androst-3 α ,17 β -diol (3 α -diol), androst-3 β ,17 β -diol (3 β -diol) and, most importantly, their glucuronidated derivatives ADT-G, 3 α -diol-G, and 3 β -diol-G.

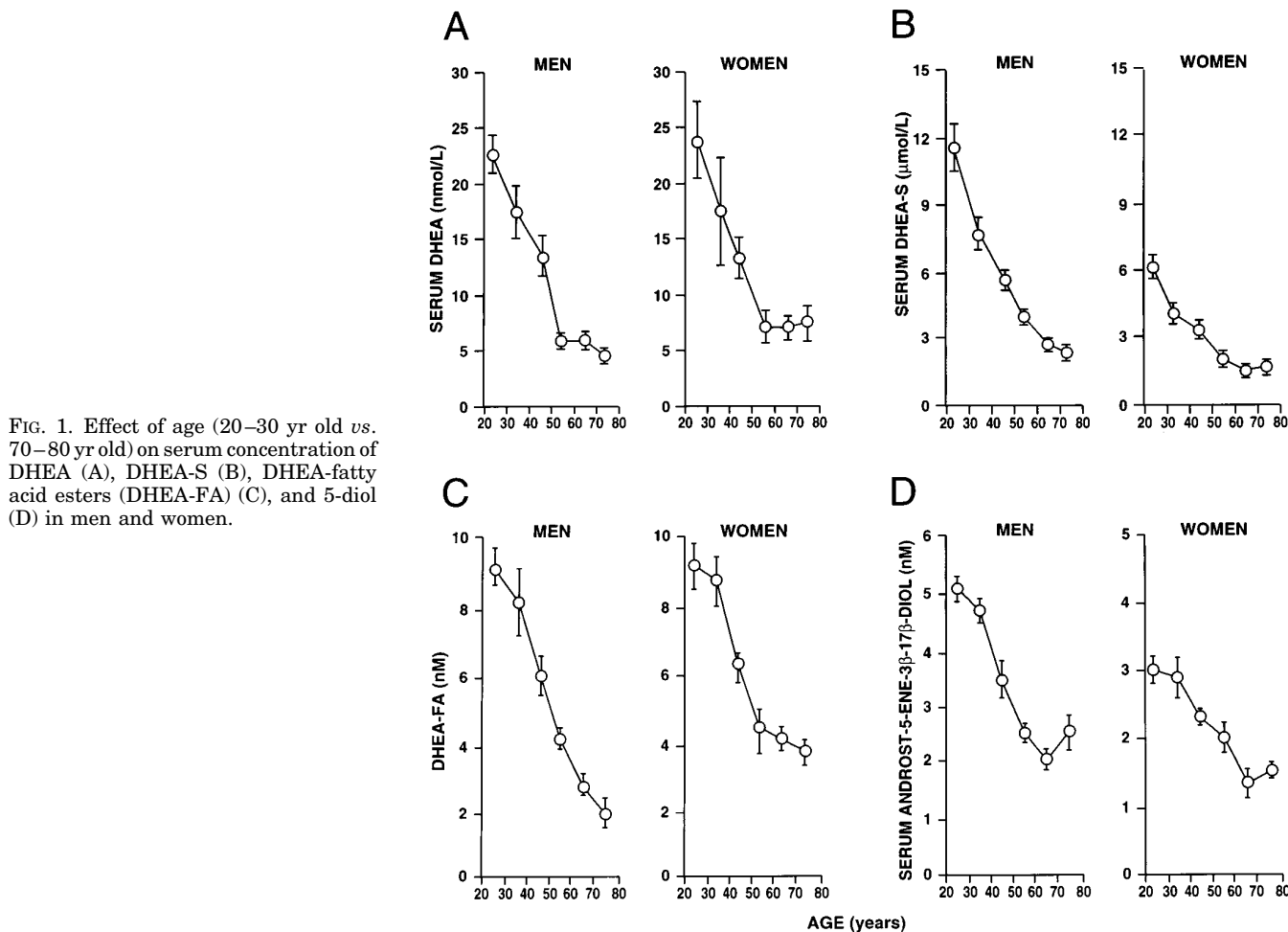


FIG. 1. Effect of age (20–30 yr old vs. 70–80 yr old) on serum concentration of DHEA (A), DHEA-S (B), DHEA-fatty acid esters (DHEA-FA) (C), and 5-diol (D) in men and women.

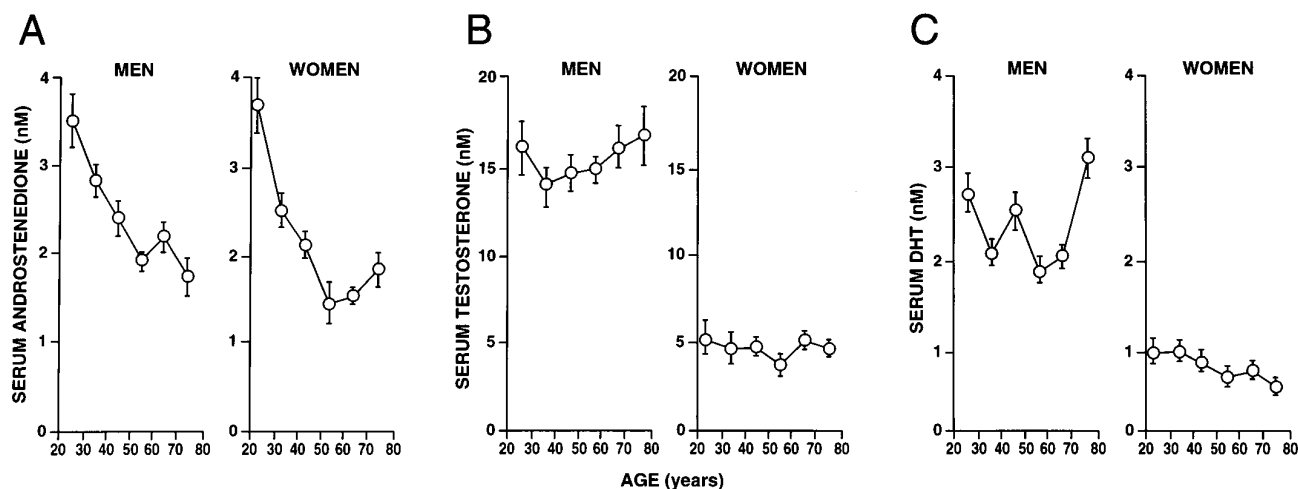


FIG. 2. Effect of age (20–30 yr old vs. 70–80 yr old) on serum concentration of 4-dione (A), testosterone (B), and DHT (C) in men and women.

ADT, the 17β -oxidized form of DHT, showed a nonsignificant trend towards a decrease with aging in both men and women (Fig. 3A). As can be seen in Fig. 3B, aging led to a maximal 30.9% decrease in the serum levels of 3α -diol of 60- to 70-yr-old men compared with 20- to 30-yr-old men, whereas in women there was no consistent trend. In contrast, serum 3β -diol decreased from 3.1 ± 0.2 nM in 20- to 30-yr-old

men to 1.8 ± 0.14 nM in 60- to 70-yr-old men (41.9% decrease) (Fig. 3C). In women, the values decreased from 1.8 ± 0.17 nM in 20- to 30-yr-old women to 1.3 ± 0.15 nM in 60- to 70-yr-old subjects.

Most importantly, serum ADT-G decreased from 81 ± 10 nM in the youngest age group of men to 22 ± 3.6 nM in the 70- to 80-yr-old group (72.8% decrease), whereas in women the

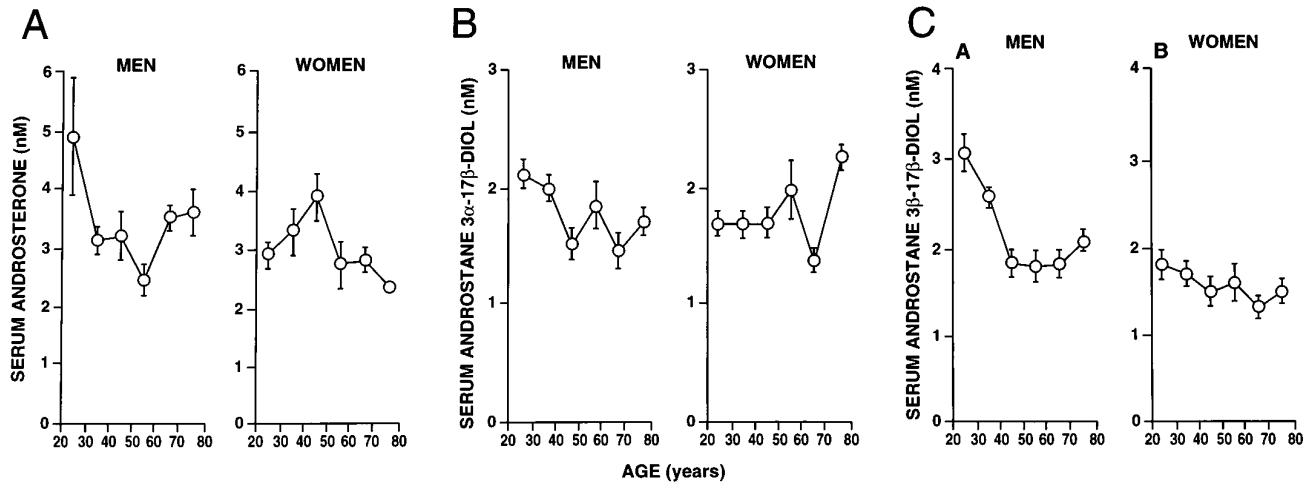


FIG. 3. Effect of age (20–30 yr old vs. 70–80 yr old) on serum concentration of ADT (A), 3 α -diol (B), and 3 β -diol (C) in men and women.

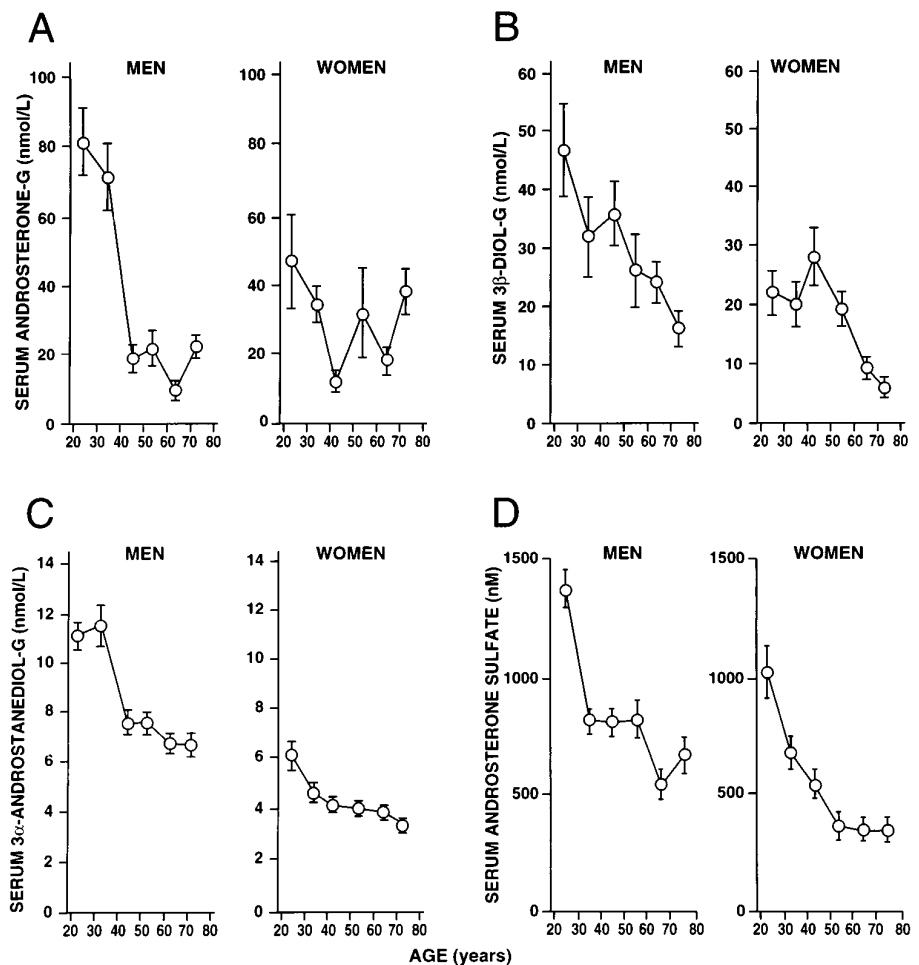


FIG. 4. Effect of age (20–30 yr old vs. 70–80 yr old) on serum concentration of ADT-G (A), 3 β -diol-G (B), 3 α -diol-G (C), and ADT-S (D) in men and women.

values of the same parameter decreased from 47 ± 14 nM in the 20- to 30-yr-old group to 18 ± 4 nM in the 60- to 70-yr-old group (61.7% decrease). There was no significant decrease in the 70- to 80-yr-old group (Fig. 4A). The serum levels of 3 β -diol-G, the second quantitatively most important metabolite of androgens, decreased from 47 ± 8 nM in the youngest group of men to 16 ± 3 nM (65.9% decrease) in the oldest

group. In contrast, in women serum 3 β -diol-G decreased from 22 ± 4 nM in the 20- to 30-yr-old group to 6.0 ± 1.7 nM in the oldest group (72.7% decrease) (Fig. 4B). Similarly, the serum concentration of the third most important metabolite of androgens, 3 α -diol-G, decreased from 11.1 ± 0.57 nM in the 20- to 30-yr-old group of men to 6.57 ± 0.5 nM in the oldest group (40.8% decrease), whereas in women serum 3 α -diol-G

decreased from 6.1 ± 0.6 nM to 3.2 ± 0.2 nM (47.5% decrease) (Fig. 4C). The serum levels of the most abundant metabolite of DHT, ADT-sulfate (ADT-S), decreased from 1380 ± 93 nM in the youngest group of men to 523 ± 49 nM (62% decrease) in the 60- to 70-yr-old group of men (Fig. 4D). In women the values in the same age groups were 1025 ± 112 nM and 344 ± 34 nM, respectively, (66.4% decrease) (Fig. 4D).

Serum E_1 and estradiol (E_2) remained approximately constant in men during the study period (Figs. 5, A and B), whereas in women the usual decline of serum E_2 was observed at menopause. It is of interest to see that serum pregnenolone (Fig. 5C) and pregnenolone-fatty acid esters (Fig. 5D) showed a decline with age somewhat comparable with that of DHEA and the metabolites of androgens. In fact, serum pregnenolone decreased from 5.5 ± 0.5 nM in 20- to 30-yr-old men to 1.2 ± 0.6 in the 70- to 80-yr-old group (78% decrease). In contrast, in women serum pregnenolone decreased from 3.8 ± 0.6 nM in the youngest age group to 2.06 ± 0.38 nM in the 70- to 80-yr-old group (45% decrease). Similarly, serum pregnenolone-fatty acid esters decreased from 11.0 ± 0.8 nM in the youngest age group to 4.3 ± 0.4 nM at 70–80 yr of age in men (60.9% decrease), whereas in women the serum concentration of the same steroid decreased from 11.0 ± 1.2 nM to a nadir of 6.2 ± 0.7 nM at 60–70 yr of age (43.6% decrease).

Discussion

It is quite remarkable that most of the important decline in circulating DHEA, DHEA-S, 5-diol, 5-diol-G, and 4-dione, as well as the conjugated metabolites of androgens, namely ADT-G, 3α -diol-G, 3β -diol-G, and ADT-S occurs between the ages of 20–30 yr and 50–60 yr, whereas smaller changes are seen after the age of 60 yr.

The above-described changes with age of the circulating levels of the metabolites of androgens are likely to be almost or exclusively caused by the dramatic decreases in DHEA and DHEA-S secretion by the adrenals during aging. As measured in a population of 2423 men, we recently found 66%, 67%, 41%, 60%, and 45% decreases during a 40-yr period of the concentration of the major C_{19} steroids, DHEA, DHEA-S, 5-diol, 5-diol-sulfate (5-diol-S), and 4-dione, respectively (1).

Although some studies show no change in the circulating levels of testosterone and DHT during aging, most report only a slight decrease in the value of these parameters (23). In a recent study performed in a large population of men, the stability of serum testosterone with age was illustrated by the finding that it decreased at a rate of 0.4% per year between the ages of 40–80 yr for a total of 16% over the 40-yr period (24). It is noteworthy that serum testosterone and DHT levels

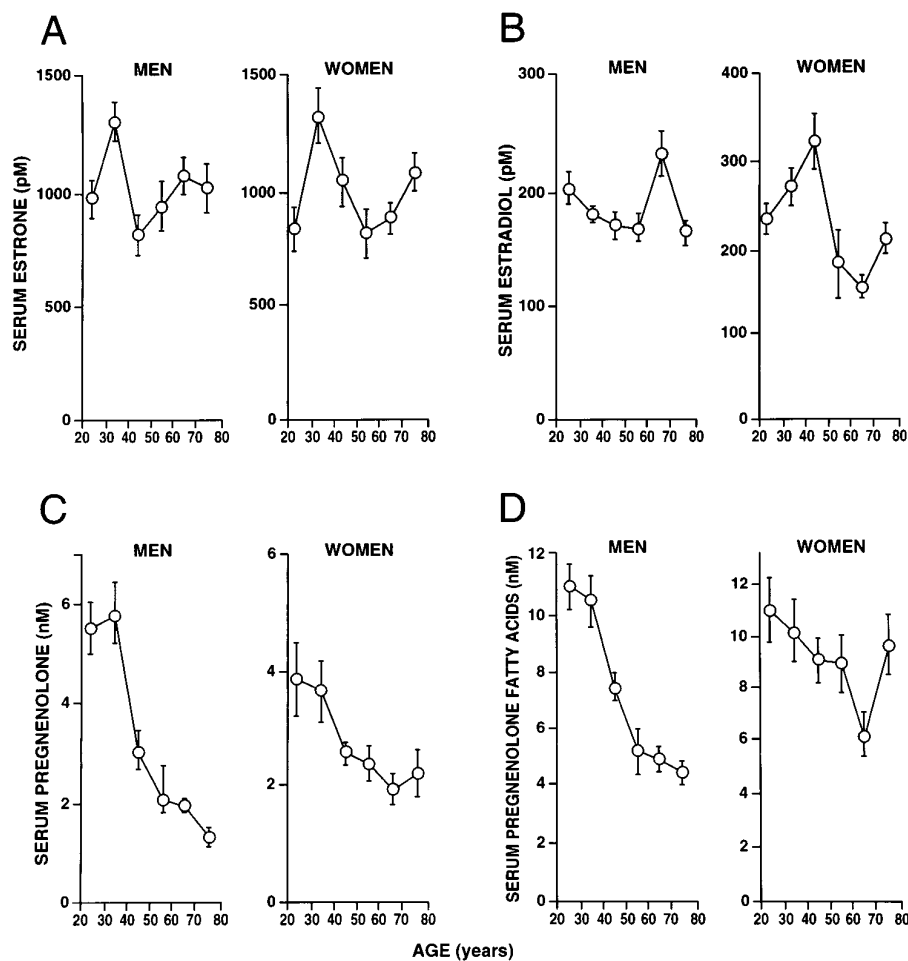


FIG. 5. Effect of age (20–30 yr old vs. 70–80 yr old) on serum concentration of E_1 (A), E_2 (B), pregnenolone (C), and pregnenolone-fatty acid esters (D) in men and women.

in women are approximately one-third of those measured in men through the ages of 20–70 yr. As mentioned above, circulating testosterone and DHT are almost exclusively of testicular and ovarian origins in men and women, respectively.

Because the concentration of serum DHEA is comparable in men and women, and serum testosterone levels decrease from approximately 15 nM to 1.5 nM following castration in men (7, 25), the low 1.5 nM levels of serum testosterone found in castrated men result from leakage into the circulation of testosterone synthesized locally from DHEA in the peripheral intracrine tissues. It is thus reasonable to suggest that approximately 3.0 nM of serum testosterone measured in women is secreted by the ovaries (67%), whereas in men approximately 13 nM of serum testosterone originates from the testes (90%). This estimate assumes that the same 1.5 nM value of serum testosterone is derived from intracrine DHEA transformation in both men and women. In agreement with these calculations, LHRH agonists have been found to reduce serum testosterone levels by 65% in women (26). However, during the early natural postmenopausal period, serum testosterone remains relatively stable (27–29). This apparent discrepancy can possibly be explained by the fact that at postmenopause, 4-dione continues to be secreted by the ovarian interstitial cells, especially by the hyperplastic stroma, resulting from high circulating gonadotropin levels (30, 31).

In the 20- to 80-yr age range, the average sum of concentrations of the unconjugated metabolites of DHT, specifically ADT, 3 α -diol, and 3 β -diol, are 3.45 nM, 1.77 nM, and 2.19 nM in men and 2.98 nM, 1.79 nM, and 1.57 nM in women, respectively. The sum of the serum concentrations of these three unconjugated DHT metabolites is thus, on average, 7.41 nM in men and 6.34 nM in women over the age range of 20–80 yr. On average, there is only a 14% lower serum level of the unconjugated DHT metabolites in women compared with men.

It seems clear from recent observations reported in both men and women that the most valid and possibly the only reliable estimate of the total androgen pool is the measurement of serum ADT-G, 3 α -diol-G, and 3 β -diol-G (1, 7, 16, 32). It was first suggested that 3 α -diol-G could be a good marker of testosterone metabolism in peripheral tissues (33, 34). The major significance of the serum concentrations of 3 α -diol-G and also ADT-G was most clearly demonstrated in men with prostate cancer treated by medical castration with an LHRH agonist or orchiectomy. In those castrated men, the serum levels of 3 α -diol-G and ADT-G decreased by only 50–70% (7, 15), whereas the plasma testosterone concentration was decreased by 90–95% (7, 16, 25).

From the data available describing the serum levels on androgens and their metabolites, as well as from direct measurement of DHT in the prostatic tissue, it can be estimated that the adrenals contribute 40–50% of total androgens in 60- to 70 yr-old men (16). The present data further suggest that measurements of serum ADT-S could be another useful parameter reflecting the total androgen pool in men and women. However, because the serum levels of ADT-G, 3 α -diol-G, 3 β -diol-G, and ADT-S change in parallel, at least during aging, measurement of the se-

rum concentration of any one of these metabolites, possibly ADT-G, can provide a reliable marker of total androgen activity in both men and women. However, because different enzymes (ADT-glucuronyltransferase and steroid sulfotransferase) catalyze the conjugation of the various androgen metabolites, differential changes in the levels of ADT-G, 3 α -diol-G, 3 β -diol-G, and/or ADT-S are possible and should be taken into account.

The small or absence of change in serum 3 α -diol in the present study as a function of age is in agreement with the data previously obtained at peripubertal age, in which the serum 3 α -diol concentration was not sensitive to marked changes in serum DHEA and testosterone levels (32). It was clear from those data that in contrast to the small changes observed in unconjugated 5 α -steroids during maturation, plasma 3 α -diol-G and ADT-G were much more sensitive to changes in the circulating levels of adrenal and testicular C₁₉ steroids.

Using the serum concentrations of ADT-G, 3 α -diol-G, 3 β -diol-G, and ADT-S as estimates of total androgens, the average sum of the serum concentrations of these conjugated metabolites of DHT are 37.5 nM, 8.47 nM, 30.2 nM, and 833.5 nM, respectively, in men compared with 32.5 nM, 4.28 nM, 17.3 nM, and 547.8 nM, respectively, in women. The average serum concentrations of ADT-G, 3 α -diol-G, 3 β -diol-G, and ADT-S measured in women between the ages 20–80 yr are thus 86.6% (ADT-G), 50.5% (3 α -diol-G), 57.2% (3 β -diol-G), and 65.7% (ADT-S) compared with those found in men of the same age. Although the metabolic clearance rates of these four metabolites are likely to show some differences between men and women, an estimate of the relative amount of total androgens in women and men calculated on the basis of the sum of the serum concentrations of these four metabolites suggests that total androgen production in women is about two-thirds or 66% of that present in men. Such data strongly suggest that androgens play a major biological role in women. The 44.5% fall in serum DHEA from 20–30 yr to 40–50 yr of age in women could well explain the bone loss and increased FSH/LH ratio that precede menopause and occur before a detectable decrease in ovarian steroidogenesis in perimenopausal women. An example of such a role of androgens could be the increasingly recognized role of androgens on bone physiology (35).

As mentioned above, the present data have major implications for a correct interpretation of serum testosterone, DHT, and E₂ concentrations in men and women, as well as serum steroid levels in humans or experimental animals treated with DHEA or intracellular inhibitors of sex steroid formation. As an example, the observation that serum testosterone and E₂ levels were not increased after treatment of male rats with finasteride led the authors to exclude the potential and even the most likely role of increased intracellular levels of testosterone (36). The present data clearly show that the increased intracellular levels of testosterone and DHT do not translate into parallel changes in circulating levels of these active androgens, and that measurement of the circulating levels of the conjugated metabolites of androgens is the most reliable marker of total androgen formation and action in the human.

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