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## Menopausal Transition Stage-Specific Changes in Circulating Adrenal Androgens

Daniel S. McConnell, PhD<sup>1</sup>, Frank Z. Stanczyk, Ph.D<sup>2</sup>, MaryFran R. Sowers, PhD<sup>1</sup>, John F. Randolph Jr, MD<sup>3</sup>, and Bill L. Lasley, PhD<sup>4</sup>

<sup>1</sup>Dept of Epidemiology, The University of Michigan, Ann Arbor, MI 48109-0404

<sup>2</sup>Dept Ob/Gyn & Prev Med, USC Keck School of Medicine, Los Angeles, CA 90033

<sup>3</sup>Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, Michigan 48109

<sup>4</sup>Center for Health and the Environment, University of California, Davis, California 95616

### Abstract

**Objective**—It is now recognized that mean circulating DHEAS concentrations in most midlife women exhibit a positive inflection starting in the early perimenopause, continuing through the early post menopause and returning to early perimenopausal levels by late post menopause. This rise in mean DHEAS is accompanied by concomitant rises in testosterone (T), dehydroepiandrosterone (DHEA), androstenedione (Adione), and an equal rise androstenediol (Adiol). These observations suggest that there is a specific relationship between the circulating levels of steroids emanating from the adrenal, declining ovarian function and stages of the menopausal transition (MT). This study was designed to test the hypothesis that the menopausal stage-specific change in circulating DHEAS is associated with concomitant changes in the circulating pattern of adrenal steroids and that some of these adrenal androgens could influence the circulating estrogen/androgen balance.

**Methods**—Stored annual serum samples (n=120) were first selected to represent four longitudinal DS profiles of individual women in order to assess and compare changes in the adrenal contribution to circulating steroids.

**Results**—Changes in mean circulating DHEAS levels in midlife women during the MT is associated with changes in mean circulating Testosterone (T), androstendione (Adione), and

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\*Corresponding Author: Daniel McConnell, DanMcC@umich.edu, Department of Epidemiology, The University of Michigan, 109 Observatory, SPH1 Rm 1867, Ann Arbor, MI 48109-2029, Telephone 734-615-9566, Fax: 734-615-9566.

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**Clinical Centers:** University of Michigan, Ann Arbor – Siobán Harlow, PI 2011, MaryFran Sowers, PI 1994-2011; Massachusetts General Hospital, Boston, MA – Joel Finkelstein, PI 1999 – present; Robert Neer, PI 1994 – 1999; Rush University, Rush University Medical Center, Chicago, IL – Howard Kravitz, PI 2009 – present; Lynda Powell, PI 1994 – 2009; University of California, Davis/ Kaiser – Ellen Gold, PI; University of California, Los Angeles – Gail Greendale, PI; Albert Einstein College of Medicine, Bronx, NY – Carol Derby, PI 2011, Rachel Wildman, PI 2010 – 2011; Nanette Santoro, PI 2004 – 2010; University of Medicine and Dentistry – New Jersey Medical School, Newark – Gerson Weiss, PI 1994 – 2004; and the University of Pittsburgh, Pittsburgh, PA – Karen Matthews, PI.

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**Central Laboratory:** University of Michigan, Ann Arbor – Daniel McConnell (Central Ligand Assay Satellite Services).

**Coordinating Center:** University of Pittsburgh, Pittsburgh, PA – Kim Sutton-Tyrrell, PI 2001 – present; New England Research Institutes, Wate rtown, MA -Sonja McKinlay, PI 1995 – 2001.

**Steering Committee:** Susan Johnson, Current Chair, Chris Gallagher, Former Chair

androstenediol (Adiol). Mean Adione and T concentrations changed the least while mean DHEAS and Adiol changed the most.

**Conclusion**—Changes in circulating steroid hormone emanating from the adrenal during the menopausal transition may be more important than the decline of ovarian function in terms of altering the estrogen/androgen balance.

### Keywords

DHEAS; androstenediol; estrogen; estrogenicity; menopause; adrenal

## Introduction

The decline in circulating dehydroepiandrosterone sulfate (DHEAS) evident from early adulthood, in both men and women, in both cross-sectional and longitudinal studies has been interpreted as an indicator of somatic aging independent of gonadal function and is utilized clinically in that capacity. The classical and expected profile of mean DHEAS is a continuous, gradual decline with advancing age. However, we have reported that, in contrast to the prevailing dogma (1–6), serum concentrations of adrenal androgens in midlife women do not always demonstrate a continuous, age-related decrease but variably exhibit a transient increase in the late perimenopause (7–9). At the present time, the underlying mechanism responsible for this increase in a circulating adrenal steroid and its relationship to the decline in ovarian function during the menopausal transition (MT) remains to be elucidated. Understanding this phenomenon may be important because of its potential as an endocrine contributor to the variability in peri-menopausal symptoms, differences in circulating steroid hormone concentrations, and/or control of adrenal function. The present study was designed to test the hypothesis that the early to late peri-menopausal change in mean circulating DHEAS is closely associated with similar changes in other circulating adrenal androgens.

## Methods

This study reports results from serum samples selected from the overall SWAN population that has been previously reported (10). Briefly, SWAN is a multi-site, longitudinal cohort study being conducted in community-based groups of women who belonged to one of 5 ethnic/racial groups. Eligibility criteria for the SWAN longitudinal cohort were: age 42 to 52 years; intact uterus and at least 1 ovary; no current use of estrogens or other medications known to affect ovarian function; at least one menstrual period in the 3 months before screening; and self-identification in one of the 5 eligible ethnic groups. Institutional Review Board approval was obtained at each study site.

A total of 120 stored serum samples were first selected to represent four longitudinal DHEAS profiles of individual women to assess annual changes in mean adrenal steroid hormones (Figure 1). Annual Profile 1 (N=30), the profile used as reference, includes data from women that exhibited the classical and expected mean DHEAS concentration at baseline followed by a subsequent gradual decline over the next two years (Table 1). In Profiles 2, 3 and 4 (N=30 each), the selected data were from women who had an initial transitory fall and then rise in circulating mean DHEAS initially from their in SWAN baseline assessment (11). Profile 2 is unique in that it has a mean DHEAS baseline that is more than 1 standard deviation (SD) below the overall population mean and has a DHEAS change profile characterized by a DHEAS fall from year 0 to year 1, with a rise in the subsequent year. Profile 3 is unique in that it has a mean baseline that is within 1 SD of the overall mean that is followed by a mean DHEAS decline the next year and followed by a rise in year 2. Profile 4 has a mean DHEAS baseline that is greater than 1 SD above the population mean and has a DHEAS change profile whereby mean DHEAS falls from year 0

to year 1 followed by a rise in year 2. Thus, Profiles 2, 3, and 4 all have profiles that represent a fall followed by a rise in mean DHEAS concentration, but all profiles link the subsequent change to a different baseline (year 0) DHEAS mean concentration (Figure 1).

Only White (59%) and African American (41%) women were included in the study. However, since circulating DHEAS concentrations at baseline are ethnically distributed, the proportion of the each ethnicity was not equal in the four profiles. The percentage of African Americans was greatest in Profile 2 (55%) and lowest in Profile 4 (22%).

### DHEAS Assay

The DHEAS assay is a competitive immunoassay run on Bayer Diagnostic's ACS-180 automated analyzer using chemiluminescent technology. This assay was developed de novo for SWAN and uses rabbit polyclonal anti-DHEAS antibody, goat anti-rabbit IgG labeled with superparamagnetic particles (PMP), and DHEAS labeled with dimethylacridinium ester (DMAE). 10  $\mu$ L of serum is required for the assay in addition to sufficient dead volume for aspiration and repeat. The assay range is 0.04–26.1  $\mu$ M (1.52 to 1020  $\mu$ g/dL) and is standardized against DHEAS obtained from Steraloids (Newport, RI). The inter-assay and intra-assay coefficients of variation were 13% and 10 %, respectively.

### Cortisol Assay

The cortisol assay is a competitive immunoassay run on Bayer Diagnostic's ACS-180 automated analyzer using chemiluminescence technology. The assay uses cortisol labeled with DMAE, a polyclonal rabbit anti-cortisol antibody, and a monoclonal mouse anti-rabbit antibody coupled to PMP. The inter-assay and intra-assay coefficients of variation were 4.8% and 3.6%, respectively.

### Testosterone Assay

The testosterone (T) assay is a competitive immunoassay modified to run on Bayer Diagnostic's ACS-180 automated analyzer using chemiluminescence technology (12). The assay uses T labeled with DMAE, a polyclonal rabbit anti-T antibody, and a monoclonal mouse anti-rabbit antibody coupled to PMP. 45  $\mu$ L of serum is required for the assay in addition to sufficient dead volume for aspiration and repeat assay determinations. The SWAN reporting range for the T assay is 0.4–3.5 nM (10–100 ng/dL). The actual assay range is 0.07–16.6 nM (2–478 ng/dL). The ACS T assay is standardized analytically and confirmed by GC-MS. The inter-assay and intra-assay coefficients of variation were 12% and 7%, respectively.

### Androstenedione and Androstenediol (Assays)

Androstenedione (Adione) and 5-androstene-3 $\beta$ ,17 $\beta$ -diol (Adiol) were analyzed in a single aliquot (0.8 ml) of serum by radioimmunoassay (RIA) methods (13,14). The analytes were extracted from plasma using hexane:ethyl acetate (3:2) and, following evaporation of the organic solvents, the extract was redissolved in isooctane and applied to a celite partition column impregnated with ethylene glycol. Adione and Adiol were eluted with isooctane and 40% toluene in isooctane, respectively. After evaporation of the eluates, each residue was reconstituted in assay buffer and appropriate aliquots were taken for RIA. Each RIA utilizes a highly specific antiserum in conjunction with an iodinated (Adione) or tritiated (Adiol) radioligand. Following an appropriate incubation period, antibody-bound vs unbound hormone were separated by use of either second antibody (Adione) or dextran-coated charcoal (Adiol). The antibody-bound hormone was then counted after centrifugation. Inter-assay and intra-assay coefficients of variation were 8% and 10%, at 460 and 1450 pg/ml, respectively, for Adione, and 13% and 11% at 460 and 1210 pg/ml, respectively, for Adiol.

### SHBG Assay

The sex hormone-binding globulin (SHBG) assay is a competitive immunoassay run on Bayer Diagnostic's ACS:180 automated analyzer using chemiluminescent technology (15). The assay was developed de novo and uses a rabbit polyclonal anti-SHBG antibody, SHBG labeled with DMAE, and goat anti-rabbit Immunoglobulin G (IgG) coupled with PMP. Forty  $\mu\text{L}$  of serum is required for the assay in addition to sufficient dead volume for aspiration and repeat. The expected values are from 20 to 130 nM. The reporting range for the SHBG assay is 10–150 nM (actual assay range: 1.95 to 250 nM). The assay is standardized against SHBG obtained from Wein Laboratories Inc. (Concord, MA). The inter-assay and intra-assay coefficients of variation were 12% and 9%, respectively.

### Estradiol Assay

The off-line estradiol (E2) assay (16) is a semi-automated, competitive immunoassay with manual steps and an off-line incubation. Standards, quality control preparations, and serum samples are pipetted into 12 $\times$ 75 polypropylene tubes by hand with a dilution of antibody reagent, and then incubated. First, DMAE-labeled E2 and rabbit anti-estradiol antibody are added to the tubes and incubated. Then all tubes are placed on Bayer Diagnostic's ACS-180 automated analyzer with monoclonal mouse anti rabbit IgG coupled to PMP for analysis. 1.0 mL of serum is required for the assay in addition to sufficient dead volume for aspiration and repeats. The SWAN reporting range for the E2 assay is 3.7–734 pM (1–200 pg/mL). The ACS E2-6 Master Curve standards are manufactured and evaluated by GC-MS. The inter-assay and intra-assay coefficients of variation were 14% and 9%, respectively.

### Data Analysis

One way ANOVA was used to test whether the values of steroids (androgens, E2) as well as SHBG in the reference DHEAS profile (Profile 1) were different than those in the DHEAS profile with unique DHEAS rises patterns (Profiles 2–4). Correlation analysis was further applied to test the strength and the direction of associations of circulating Adione, Adiol, and T with DHEAS at the levels of baseline. Hormone values were log transformed prior to analysis. A p value of less than 0.05 was considered to be statistically significant. Data analysis was done using StatView Version 5.0.1.0 (1998, SAS Institute, Inc.) and SAS version 8.0. Simple linear regression was used to illustrate the relationships between T, DHEAS, Adione and Adiol. In the regression analyses all hormone concentrations were log transformed prior to analysis and then back transformed to remove skewness.

### Results

While there was no statistically significant difference in comparing the age of the reference Profile 1 women to characteristics of the other 3 profiles, there was a nonsignificant trend that the reference women had a greater BMI as well as were more likely to be perimenopausal than premenopausal (Table 1). Women in the reference Profile 1 were more likely to report having hot flashes in the two weeks prior to the baseline examination as compared to the other profiles.

The four distinct hormone profiles contrast an expected continuing gradual decline in mean DHEAS as shown in Profile 1 (Figure 1 and Table 2) to the main patterns of increase and decline that were captured in the larger SWAN study. These patterns most likely represent the same basic physiology mechanism but appear as different patterns because the women were recruited at different times relative to an inflection in DHEAS production.

Baseline mean DHEAS were associated with different androgen profiles using regression analysis. The DHEAS decline and increase were attended by concomitant changes in Adiol

and, to a lesser degree, T, and Adione (Figure 2). Further, a consistently stronger correlation was found between DHEAS and Adiol (0.71,  $p < 0.05$ ), irrespective of the baseline level of DHEAS compared to T (0.20,  $p < 0.05$ ) and Adione (0.28,  $p < 0.05$ ). Circulating mean cortisol concentrations were not correlated with DHEAS and there was no difference in mean cortisol concentrations among the different profiles (data not shown).

## Discussion

A menopausal stage-specific rise in mean serum DHEAS is observed in most women between the early peri-menopause and early post menopause stage of the MT (8), which also occurs following bilateral salpingo-oophorectomy during the early perimenopause (9). This change in DHEAS is accompanied by simultaneous and similar changes in circulating adrenal androgens but not cortisol. Biologically, an increase in these androgenic steroids could be important for women approaching menopause given the fact that DHEAS, resulting in a rise in DHEA, provides a reservoir of substrate for peripheral conversion into other bioactive steroids. Interestingly, one of these metabolites, Adiol, has both inherent estrogenic and androgenic activity (17, 18) and possibly contributes directly to the circulating estrogen pool and alterations in the circulating estrogen to androgen ratio (19). This previous report showed the relationship between the four adrenal androgens, DHEAS, T, Adiol and Adione (19) and we speculate that these hormone would move together; the present data show that increasing circulating levels of DHEAS are accompanied by concomitant increases in Adiol, Adione and T in peri-menopausal women. This is consistent with the literature, which indicates that circulating DHEAS is a reliable index of total androgens in women (20).

Circulating Adiol concentrations were more robustly correlated to DHEAS during all segments of the perimenopausal dynamics of DHEAS as compared to Adione and T in the longitudinal analysis presented here. This observation of a strong, positive correlation between DHEAS and Adiol is consistent with the concept that Adiol is a peripheral conversion product of DHEA. It is also possible that it is a direct secretory product of the adrenal. The lack of correlation between DHEAS and E2, however, does not support the contention that peripheral metabolism of DHEA contributes significantly to circulating E2. The absence of data demonstrating the conversion of exogenous DHEA to Adiol concentrations similar to those observed in this study also suggests that much or most of the circulating Adiol that occurs during the MT is a result of direct secretion by the adrenal glands. While Adiol is a weak estrogen agonist compared to E2, the serum concentrations of Adiol in mid-life women are substantially higher than levels of E2 during the menopausal transition in most women (19). The five-fold or greater range of circulating Adiol levels, from 689 pM (200 pg/mL) to greater than 3.8 nM (1100 pg/mL), presents the possibility that some women may experience estrogen action from a sufficient increase in Adiol. In contrast, the range of circulating E2 concentrations is only 3 fold (20–60 pg/mL) when measured during the early follicular phase of the menstrual cycle. Therefore, during the MT, the between-woman difference in circulating Adiol concentrations exceeds the between-woman difference in E2 by up to one thousand fold, potentially providing a more variable source of estrogenic, albeit weak, support.

Recent interest has arisen in identifying the direct effects of steroid hormone metabolites that may activate steroid hormone receptors (18). The present data provide additional and direct evidence for a potential role of steroids emanating from the adrenal in modulating symptoms and health outcomes of mid-life women. The circulating levels for Adione and T increased only two and three fold, respectively, and only a modest statistically significant difference was found in the mean Adione concentrations across the total range of DHEAS concentrations. This modest increase in Adione and T in association with the robust rise in

DHEA supports the concept that while there is likely an increased conversion of DHEA to Adione and T in women during early-to-late MT, the much greater rise in DHEA suggests that changes in adrenal secretion rates rather than just peripheral conversion may be important in the support of sex-steroid sensitive tissues. To be clear, this report does not demonstrate a direct secretion of steroids by the adrenal gland is responsible, but in the face of no evidence in the literature to suggest changes in metabolism or clearance rates could account for such concentration changes, we interpret the observed increase as due to increased production rate. Thus, increased Adiol production (with inherent estrogenic activity), rather than an elevation of DHEA as a prohormone, suggests an alternative explanation for the positive physiological effects that have previously been attributed primarily to endogenous DHEA.

The grouping of women based on initial circulating DHEAS concentrations created trajectory groups that were ethnically divergent. This is to be expected, as previous reports have demonstrated ethnic differences in DHEAS in mid-aged women (8, 21), in which Whites have higher, and African Americans lower, baseline DHEAS. Despite this apparent initial, ethnic based difference in baseline DHEAS, the relative change in the complement of adrenal androgens during the MT was similar for all four trajectories. Thus, the absolute peak concentrations of adrenal androgens remain ethnically distinct.

This study failed to detect a clear pattern in circulating cortisol with respect to DHEAS profiles, which is consistent with a separate and selective acceleration of the  $\Delta 5$ -C19 androgen synthetic pathway and argues against a “spill-over” resulting from an increased ACTH drive. Increased ACTH drive would have selective preference for the  $\Delta 4$  pathway, resulting in increases in Adione and T concentrations, which is opposite to that observed in the present study. The present data also argue that the observed increase in adrenal secretory activity is not a result of a stress response, either specific to the perimenopause or independent of it. Although cortisol was measured in these profiles, these results should be interpreted with caution because samples for the SWAN cohort were only collected between 6 and 10 am.

## Conclusion

The data presented herein illustrate that there are wider between-woman differences in circulating adrenal steroids during the MT and immediately following menopause than previously appreciated. Since approximately 85% of all women have some rise in DHEAS during the menopausal transition (Crawford et al., 2009), then we expect that the increased contribution of adrenal steroids as demonstrated here is a common event for most women. While circulating E2 concentrations are known to decline in women over the course of the MT (12), the current data demonstrate that a modest decline in circulating levels of E2 in the late peri-menopause is associated with a much broader range of circulating levels of Adiol and DHEA. Thus the change in steroids emanating from the adrenal during the MT may be an important contributor to the overall change in sex steroid hormone balance over the MT and it may be a better indicator of phenotype than the smaller decline in ovarian steroid production. These observations raise the question as to the possibility that differences in adrenal steroid levels may predict difference in symptoms and health outcomes. However, since the rise in adrenal steroids was recognized only when a large number of longitudinal samples were evaluated (Crawford, et al., 2009), it is unlikely that parallel changes in symptoms or health outcomes would be detected in this relatively small cross-section investigation. Future longitudinal studies are planned that will address these interesting possibilities.

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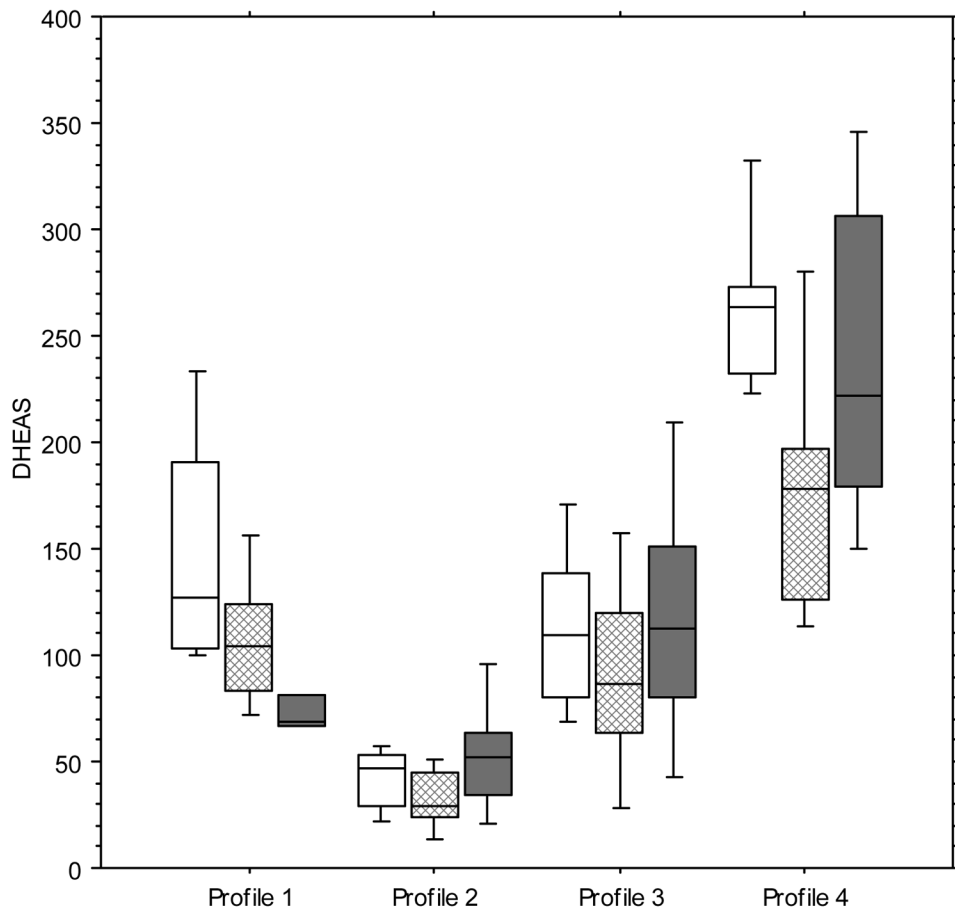
We thank the study staff at each site and all the women who participated in SWAN

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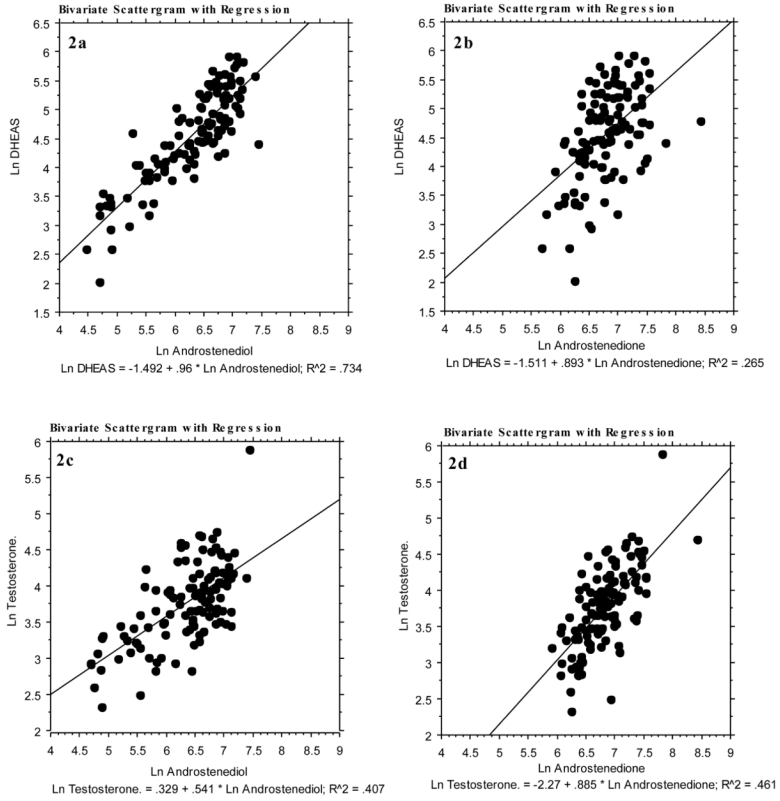
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**FIG. 1.** Serum DHEAS patterns for the selected study cohort. Reference profile 1 (n = 30) depicts the mean and range of DHEAS at baseline with the classical anticipated continued decline from year 0 (open boxes) to year 1 (hatched boxes) and year 2 (solid boxes). Profiles 2 (selected to represent the baseline mean of >1 SD below the  $\bar{x}$  of reference DHEAS with a subsequent increase; n = 30), 3 (at the  $\bar{x}$  of reference DHEAS; n = 30), and 4 (>1 SD above the  $\bar{x}$  of the reference DHEAS with an immediate decline and recovery; n = 30) illustrate the samples in which there was the transitory fall and then rise, a profile that has been uniquely reported in SWAN enrollees. Boxes represent the SEM and bars the range. DHEAS, dehydroepiandrosterone sulfate.



**FIG. 2.** Linear regressions of the four hormones described in this study (Ln DHEAS and Ln T versus Ln Adiol and Adione). Panels 2a and 2b show Ln DHEAS versus Ln Adiol (n = 119,  $P < 0.0001$ ) and Ln Adione (n = 119,  $P < 0.0001$ ), respectively. Panels 2c and 2d show Ln T vs Ln Adiol (n = 117,  $P < 0.0001$ ) and Ln Adione (N = 117,  $P < 0.0001$ ), respectively. Some samples were excluded for lack of T, Adiol, or Adione assay results. DHEAS, dehydroepiandrosterone sulfate; T, testosterone; Adiol, androstenediol; Adione, androstenedione.

**Table 1**

Demographic and menopausal status characteristics of the women exhibiting the four profiles determined by baseline DHEAS values and changes in subsequent annual DHEAS values (N=30)<sup>a</sup>.

Characteristic	Baseline DHEAS Reference Profile 1 (N=30) Mean (SD)	Baseline DHEAS < 1 SD of DHEAS Profile 2 (N=30) Mean (SD)	Baseline DHEAS at the Mean Profile 3 (N=30) Mean (SD)	Baseline DHEAS > 1 SD of Mean Profile 4 (N=30) Mean (SD)	p-value for overall subgroup difference
Age (years)	45.3 (2.5)	46.9 (3.6)	46.6 (2.4)	46.6 (2.8)	0.62
Body mass index (kg/m2)	33.6 (9.1)	31.8 (6.5)	30.8 (8.7)	29.9 (7.4)	0.81
Ethnicity:	Percent (N)	Percent (N)	Percent (N)	Percent (N)	
African American	50% (5)	50% (5)	40% (4)	20% (2)	0.51
White	50% (5)	50% (5)	60% (6)	80% (8)	
Menopause status:					0.15
Premenopausal	20 (2)	40 (4)	60 (6)	70 (7)	
Early perimenopausal	80 (8)	60 (6)	40 (4)	30 (3)	
Hot flashes in past 2 weeks (yes)	50 (5)	40 (4)	0.0 (0)	0.0 (0)	0.004
Current smoking (yes)	40 (4)	40 (4)	0.0 (0)	10 (1)	0.06
Perceived stress	9.0 (2.4)	6.6 (2.7)	8.0 (2.4)	7.6 (2.7)	0.24
Difficulty paying for basics (yes):					0.07
Very hard	10 (1)	20 (2)	0.0 (0)	0.0 (0)	
Somewhat hard	50 (5)	0.0 (0)	20 (2)	30 (3)	
Not hard at all	40 (4)	80 (8)	80 (8)	70 (7)	

<sup>a</sup>The Reference Profile shows a DHEAS fall in subsequent years, while Profiles 2–4 show a rise in the subsequent year and then fall.

Hormonal characteristics of the women exhibiting the four profiles determined by baseline DHEAS values and changes in subsequent annual DHEAS values (N=30)<sup>a</sup>.

**Table 2**

Characteristic	Baseline DHEAS Reference Profile 1 (N=30) Mean (SD)	Baseline DHEAS < 1 SD of Mean Profile 2 (N=30) Mean (SD, p profile 2 and 3)	Baseline DHEAS at Mean Profile 3 (N=30) Mean (SD)	Baseline DHEAS > 1 SD of Mean Profile 4 (N=30) Mean (SD, p profile 3 and 4)	p-value for overall subgroup difference
Androstenedione (pg/mL)	1515.1 (1174.0)	973.2 (446.2, p=0.169)	927.6 (350.6)	1188.7 (344.7, p=0.210)	0.19
Androstenediol (pg/mL)	865.4 (205.3)	287.7 (139.1, p=0.117)	703.6 (327.0)	1023.8 (266.5, p=0.056)	< 0.0001
DHEAS (ug/dL)	144.6 (49.5)	41.6 (14.1, p=0.004)	112.2 (39.2)	265.1 (44.3, p=0.001)	< 0.0001
SHBG (nM)	46.9 (17.6)	48.7 (28.1, p=0.560)	42.3 (22.8)	32.2 (11.4, p=0.386)	0.36
Testosterone (ng/dL)	61.7 (22.9)	34.6 (23.9, p=0.553)	51.0 (32.4)	63.0 (24.1, p=8.828)	0.008
Estradiol (pg/mL)	81.2 (36.3)	79.9 (72.3, p=0.675)	59.3 (49.4)	66.4 (86.9, p=0.383)	0.26

<sup>a</sup>The Reference Profile shows a DHEAS fall in subsequent years, while Profiles 2–4 show a fall in the subsequent year and then rise. P values are provided to illustrate the significance between the mean (Profile 3) and < 1SD below the mean (Profile 2) and between the mean (Profile 3) and > 1SD above the mean (Profile 4).