CLINICAL STUDY

A longitudinal study of dehydroepiandrosterone sulphate (DHEAS) change in older men and women: the Rancho Bernardo Study

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Abstract

Objective: To examine the age-related and sex-specific rates and determinants of change in endogenous dehydroepiandrosterone sulphate (DHEAS) levels in older community-dwelling adults, taking into account regression to the mean.

Design: Prospective cohort study from Rancho Bernardo, California, USA.

Methods: Plasma for DHEAS was collected at baseline and 10–14 years later from 242 men and 207 postmenopausal women (age range, 60–77 years), who were not taking hormone therapy or corticosteroids. Age-related and sex-specific changes in DHEAS were calculated according to four different definitions of change: absolute change, annual percentage change, change exceeding 10% of baseline and change exceeding that expected from statistical regression to the mean. Determinants of DHEAS change were assessed using regression analyses.

Results: Baseline DHEAS levels were higher for men than women (age-adjusted means, $1.37 \pm 0.73 \mu\text{g/ml}$ ($3.72 \pm 1.98 \mu\text{mol/l}$) vs $0.73 \pm 0.48 \mu\text{g/ml}$ ($1.98 \pm 1.30 \mu\text{mol/l}$) respectively, $P < 0.0001$), with more pronounced declines observed in men ($-4.0\%/ \text{year}$) compared with women ($-2.1\%/ \text{year}$; $P < 0.001$). Some 28% of women and 5% of men had DHEAS levels that increased or stayed the same according to a 10% definition of change. When regression to the mean artifact was accounted for, only 15% of women and 5% of men showed true increases in DHEAS. In both sexes, baseline DHEAS levels accounted for three-quarters of the variability in absolute DHEAS change over time, with higher baseline levels resulting in greater loss. Sex, baseline weight, age and smoking status were significantly associated with DHEAS change in univariate models; only sex remained independently associated with DHEAS change in multivariate analyses, with men showing greater annual declines than women (estimated coefficient = 0.006, $P = 0.008$). **Conclusion:** In this sample, over 30% of the observed changes in DHEAS could be attributed to regression to the mean. Potential underlying mechanisms of change in DHEAS levels, and sex differences, are discussed.

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Introduction

Dehydroepiandrosterone (DHEA) and its sulphate form (DHEAS) are the most abundant endogenous sex hormones in ageing men and women (1, 2). DHEAS is produced in the adrenal cortex and is converted to androgens and oestrogens in peripheral tissues (2, 3). It provides close to 100% of active oestrogens in postmenopausal women, and up to 50% of androgens in older men and women (2, 4, 5).

Cross-sectional studies have shown significant age-related declines in circulating levels of DHEAS among older men and women (4, 6); however, longitudinal studies suggest that DHEAS levels may actually

increase in 10–40% of older adults (7–9). This discrepancy remains largely unexplained. It could be due to the way that change is defined in each of the studies or to the influence of a regression to the mean phenomenon (8, 9). Regression to the mean describes the tendency of high or low values of a biological marker to return towards the mean upon re-testing (10–13). Biological variability within the individual and measurement error are thought to underlie this statistical phenomenon. Regression to the mean has been studied in relation to blood pressure changes over time (14), but the concept applies equally to changes in serum hormone levels. The importance of regression towards the mean is that it can result in overestimation

or underestimation of true change, and lead to erroneous conclusions about the natural progression of biological markers such as DHEAS over time. The effect of regression toward the mean in studies of DHEAS change has never formally been tested.

The physiological significance of DHEAS change and the factors that determine DHEAS levels also remain unclear. Converging evidence suggests that low DHEAS levels may have important but different roles in men and women with regard to cardiovascular mortality (15–22), cognitive decline (15, 23–29), accelerated bone loss (30–32), depression (23, 33) and cancer (34–37). A better understanding of the factors that influence the magnitude and direction of DHEAS change in ageing men and women is required to characterize the natural progression and clinical significance of this hormone. The present study aims to clarify the age-related and sex-specific rates and determinants of change in DHEAS levels in community-dwelling older adults, taking into account a possible regression to the mean phenomenon.

Subjects and methods

Subjects

Subjects are 449 men and women aged 60 years and older with baseline and 10- to 14-year follow-up DHEAS measurements who were enrolled in a population-based longitudinal cohort study of heart disease risk factors in the geographically defined community of Rancho Bernardo, California, USA. All women were postmenopausal. Subjects were excluded if they were taking oestrogens, progestins or corticosteroids at baseline (1972–1974) or follow-up (1984–1987).

DHEAS measurements

Venous blood for DHEAS assay was obtained from each subject at baseline and at a 10- to 14-year follow-up visit. Blood samples were obtained between 0730 and 1100 h, after a requested 12-h fast, and the plasma was separated and frozen at -70°C . Between 1985 and 1986, the baseline plasma specimens were first thawed and DHEAS was measured by direct RIA in an endocrinology research laboratory at the University of California, San Diego; 10–14 years later (1984–1987), blood from a second visit was obtained and frozen for future analysis. Between 1992 and 1994, first-thawed plasma from the second visit was assayed for DHEAS in the same laboratory, by the same technician, using the same RIA technique. The sensitivity and intra-assay and inter-assay coefficients of variation for the DHEAS assays were $0.15\text{ }\mu\text{g/ml}$ ($0.41\text{ }\mu\text{mol/l}$) and 4.2% and 8.8% respectively.

Health parameters

A standardized health questionnaire including questions about basic demographics and current cigarette smoking (yes/no) was completed by the participants at each visit. Height (m) and weight (kg) were measured with the participants wearing lightweight clothing without shoes. Body mass index (BMI) ($\text{weight (kg)}/\text{height (m)}^2$) was used as an estimate of obesity. Weight change and BMI change were calculated as weight and BMI measured at the 1984–1987 visit subtracted from weight and BMI measured at the 1972–1974 visit respectively. Vital status was determined for 99.9% of the cohort during a 15-year follow-up. Each participant gave written informed consent to participate at each clinic visit.

Defining change in DHEAS

Absolute changes in intra-individual DHEAS levels across the 10- to 14-year study period were calculated by subtracting the DHEAS level measured at the initial visit from the DHEAS level measured at the follow-up visit. The percentage change – or relative change – in DHEAS was calculated as an individual's absolute change in DHEAS divided by their baseline DHEAS level. We studied the distribution of change beyond what might be accounted for by measurement error and biological variability according to two separate definitions of 'real' change. The first was an intra-individual increase or decrease in DHEAS of greater than 10% from baseline over the follow-up period. This 10% value was based on the inter-assay coefficient of variation and other estimates of DHEAS stability and intra-individual reproducibility over time in older adults (38, 39). A second calculation of change due to measurement error and biological variability was carried out by simulating DHEAS follow-up values based entirely on statistical regression to the mean criteria. For each participant, we simulated the expected change in DHEAS due to regression to the mean, subtracted the observed baseline measure from this value, and then compared the expected change in DHEAS due to regression to the mean with the absolute change in DHEAS observed during the study period. To simulate regression to the mean follow-up values of DHEAS, we used the formula according to Streiner (11):

$$T_2 = \bar{X} + r(T_1 - \bar{X})$$

where: T_1 is the person's initial DHEAS level at baseline; T_2 is the simulated follow-up score; $\bar{X}$ is the sex-specific mean for the observed baseline and follow-up DHEAS values; r is the sex-specific correlation between the observed baseline and follow-up measures. Participants were classified as having a decrease in DHEAS if their absolute change in DHEAS was less than (e.g. was algebraically more negative than) the expected change due to

regression to the mean; non-decliners were those who showed increases in DHEAS as defined by an absolute change in DHEAS that exceeded (e.g. was algebraically more positive than) the expected change due to regression to the mean.

Fifteen participants at baseline and 36 at follow-up had DHEAS levels that were below the assay sensitivity. These participants were assigned the minimum threshold value of 0.15 µg/ml (0.41 µmol/l). Nine participants had DHEAS levels below the assay sensitivity at both baseline and follow-up. According to the 10% cut-off distribution of change criteria, these subjects were classified as having stayed the same.

Statistical analysis

Statistical analyses were conducted using the SAS software package (version 8.2, SAS Institute, Cary, NC, USA). Subject characteristics were summarized according to basic descriptive statistics for means and proportions. To ascertain response bias, Student's *t*-tests and χ^2 analyses were used to compare baseline characteristics across sex and age strata for participants who came to both visits versus those who had died prior to the second clinical visit or who had survived to the second visit but who did not have repeat DHEAS measurements. Age-adjusted mean baseline DHEAS levels for the different groups were computed using analysis of covariance PROC GLM.

Correlations between baseline and follow-up DHEAS levels as well as with DHEAS change were computed using PROC CORR. The sex- and age-adjusted partial correlation of baseline and follow-up DHEAS measures was calculated using the PARTIAL option to determine the strength of this relationship when the effects of sex and age were removed. Changes in DHEAS according to different definitions of change were calculated using basic descriptive statistics. The percentage change in DHEAS that could be accounted for by regression to the mean was calculated as $100(1 - \text{partial } r)$ where partial *r* is the sex- and age-adjusted partial correlation of baseline and follow-up DHEAS measures in this sample (12).

Determinants of annual absolute change in DHEAS in the study group were evaluated using linear regression and determinants of categorical change were evaluated using logistic regression. Participants whose DHEAS levels declined by greater than 10% were compared with those whose levels stayed the same or increased by 10%. In a separate analysis, participants whose change in DHEAS exceeded what was expected due to regression to the mean were compared with those whose DHEAS change was less than what was expected due to regression to the mean. Baseline DHEAS levels, age, sex, BMI, baseline body weight, weight change, change in BMI and baseline smoking status were evaluated as potential predictors of DHEAS change. Predictors were first tested individually,

using univariate regression. Predictors that emerged as significant in univariate testing ($P < 0.1$) were then introduced into multivariate models. Logistic regression analyses were used to differentiate women whose DHEAS levels increased or stayed the same from those whose DHEAS levels decreased (this was not possible for men because few had DHEAS levels that increased or stayed the same). To test whether increases in DHEAS were associated with lower initial DHEAS levels, a DHEAS cut-off value corresponding to the lowest quartile of baseline DHEAS in women (0.38 µg/ml (1.03 µmol/l)) was selected as a predictor. Results are presented as regression coefficients (beta estimates) and proportion of explained variance (R^2) of the linear models, and as odds ratios with 95% confidence intervals (OR $\pm$ 95% CI) for logistic models.

Results

Demographic characteristics

Among 1279 potentially eligible subjects who had DHEAS measurements at baseline, 444 died prior to the 12-year follow-up; among those who survived, 386 did not return for repeat DHEAS testing. The remaining 449 subjects are the focus of this report. Of these, 242 were men (mean age, 67.1 ± 3.7 years; range, 60–77 years) and 207 were women (mean age, 66.7 ± 4.1 years; range, 60–76 years). The characteristics of the 449 study participants compared with the group of 444 subjects who died, and the 386 subjects who survived but did not have repeat DHEAS measurements, are presented in Table 1. As shown, those who died were older and more likely to smoke than the study participants. Although baseline DHEAS levels were significantly lower in men (but not women) who died prior to the follow-up visit, this difference was not significant after adjusting for age using analysis of covariance.

DHEAS change over time

Age-related and sex-specific rates of absolute, relative and 'real' changes in DHEAS over the 10- to 14-year follow-up (mean 11.8 ± 0.8 years) are presented in Tables 2 and 3. At baseline and at follow-up DHEAS levels were higher for men than women in every age group ($P < 0.0001$). Annual rates of decline were consistently more pronounced in men than in women (age-adjusted means, -4.0% vs -2.1% respectively; $P < 0.0001$). Relative rates of change remained stable for men and women across age groups. Overall, only 5% of men showed a stable or increasing level of DHEAS over time according to both the $\pm 10\%$ criteria for change as well as the regression to the mean criteria. In contrast, DHEAS levels were stable or increased in 28% of women according to the $\pm 10\%$

Table 1 Baseline characteristics of subjects included and excluded from the study.

	Study population (n = 449)		Alive but no DHEAS follow-up (n = 386)		Died prior to 12-year follow-up (all cause mortality; n = 444)	
	Men (n = 242)	Women (n = 207)	Men (n = 185)	Women (n = 201)	Men (n = 280)	Women (n = 164)
Mean age $\pm$ s.d.	67.1 $\pm$ 3.7	66.7 $\pm$ 4.1	68.1 $\pm$ 5.0*	68.4 $\pm$ 5.2*	70.3 $\pm$ 5.0*	71.5 $\pm$ 5.7*
Age range (years)	60–77	60–76	60–79	60–82	60–82	60–88
Weight $\pm$ s.d. (kg)	77.4 $\pm$ 10.4	63.7 $\pm$ 10.3	77.5 $\pm$ 10.0	61.8 $\pm$ 10.0	76.0 $\pm$ 10.2	62.0 $\pm$ 10.7
BMI $\pm$ s.d.	25.6 $\pm$ 2.9	24.8 $\pm$ 3.8	25.8 $\pm$ 2.8	24.5 $\pm$ 3.9	25.4 $\pm$ 2.9	24.7 $\pm$ 4.2
Current smoker (%)	12.4	13.0	15.7	24.4*	17.5	22.0*
Mean baseline DHEAS (μ g/ml)	1.36	0.74	1.31	0.76	1.18*	0.71
(μ mol/l)	(3.69)	(2.00)	(3.55)	(2.06)	(3.20)	(1.93)
Mean age-adjusted baseline DHEAS	1.29	0.71	1.29	0.76	1.26	0.75
(μ g/ml) (μ mol/l)	(3.50)	(1.93)	(3.50)	(2.06)	(3.42)	(2.04)
DHEAS range (μ g/ml)	0.20–4.93	0.15–3.00	0.24–3.80	0.15–3.51	0.15–4.95	0.15–3.32
(μ mol/l)	(0.54–13.38)	(0.41–8.14)	(0.65–10.31)	(0.41–9.53)	(0.41–13.43)	(0.41–9.01)

*P < 0.05 compared to study population.

criteria, and in 15% according to the regression to the mean criteria.

Accordingly, only 10% of the total sample (5% of men and 15% of women) showed 'real' increases in DHEAS when regression to the mean was accounted for.

Correlations between baseline and follow-up DHEAS levels and DHEAS change

Baseline DHEAS levels were highly correlated with follow-up DHEAS levels ($r=0.69$ for men and 0.73 for women; $P < 0.0001$ for both) and with DHEAS change ($r = -0.87$ for men and -0.72 for women; $P < 0.0001$ for both). The sex- and age-adjusted partial correlation between baseline and follow-up DHEAS levels was 0.69 . The percentage change in DHEAS that could be accounted for by regression to the mean was calculated to be 31%. Analyses were repeated excluding subjects whose DHEAS levels at baseline or follow-up fell below the assay sensitivity, with no measurable changes in the results.

Determinants of DHEAS change

Age, sex, baseline DHEAS, baseline weight and smoking status were significantly associated with annualized change in DHEAS in univariate analyses (Table 4). Only baseline DHEAS and sex remained independently associated with DHEAS change in multivariate models. The relationship between baseline weight and DHEAS change was confounded by sex, and the effects of age and smoking status on DHEAS change no longer remained significant when adjusted for baseline DHEAS levels. Baseline DHEAS was clearly the most important predictor, accounting for 74% of the variability in DHEAS change. Figure 1 illustrates the association of higher baseline values of DHEAS with larger negative

change, and lower baseline values with smaller declines or even increases in DHEAS.

Logistic regression analyses were used to differentiate those women whose DHEAS levels increased or stayed the same from those whose DHEAS levels decreased. Using the $\pm 10\%$ criteria for change, having DHEAS levels in the lowest quartile of baseline DHEAS was the only significant predictor of an increase in DHEAS levels (for baseline DHEAS levels less than or equal to $0.38 \mu\text{g/ml}$, OR 2.41, 95% CI 1.21–4.81). Smoking status – and increases in body weight, BMI and age – were not associated with the direction of DHEAS change. In analyses looking only at women in whom DHEAS increases exceeded those attributable to regression to the mean, low baseline DHEAS levels did not remain a statistically significant predictor of 'real' change.

Determinants of the direction of DHEAS change in older men were not possible in this study as there were insufficient numbers of men in whom DHEAS levels did not decrease.

Discussion

Data from this longitudinal study show that plasma DHEAS levels continued to decline in most participants after age 60, but with marked individual variability in both sexes. Declines in DHEAS were more pronounced and nearly universal in men while women were more likely to show smaller declines or have an increase in DHEAS levels. Baseline DHEAS was the most important predictor of DHEAS change, with sex being the only other independent predictor. Smoking status, age and baseline weight possibly exerted small additional effects on DHEAS change through their effects on baseline DHEAS.

These findings confirm the overall age-related trend of lower circulating DHEAS levels observed in

Table 2 DHEAS change over a 12-year time period in men.

Age group (years)	n	Mean baseline DHEAS ($\mu\text{g/ml}$) ($\mu\text{mol/l}$)	Range of baseline DHEAS ($\mu\text{g/ml}$) ($\mu\text{mol/l}$)	Mean 10- to 14-year follow-up DHEAS ($\mu\text{g/ml}$) ($\mu\text{mol/l}$)	Range of 10- to 14-year follow-up DHEAS ($\mu\text{g/ml}$) ($\mu\text{mol/l}$)	10- to 14-year difference in DHEAS ($\mu\text{g/ml}$) ($\mu\text{mol/l}$)	Annual percentage change DHEAS	Distribution of change by $\pm 10\%$			Distribution of change by regression to the mean criteria	
								Decrease (%)	Same (%)	Increase (%)	Decrease (%)	Increase (%)
60–64	61	1.48 $\pm$ 0.64 (4.03 $\pm$ 1.75)	0.45–3.32 (1.22–9.01)	0.69 $\pm$ 0.37 (1.89 $\pm$ 1.00)	0.15–1.57 (0.41–4.26)	–0.78 $\pm$ 0.50 (–2.13 $\pm$ 1.37)	–4.3 $\pm$ 2.0	95	2	3	95	5
65–69	121	1.36 $\pm$ 0.69 (3.70 $\pm$ 1.89)	0.20–4.35 (0.54–11.81)	0.67 $\pm$ 0.38 (1.82 $\pm$ 1.05)	0.15–1.96 (0.41–5.32)	–0.66 $\pm$ 0.49 (–1.81 $\pm$ 1.35)	–4.1 $\pm$ 1.9	97	2	1	96	4
70–74	52	1.29 $\pm$ 0.92 (3.50 $\pm$ 2.52)	0.24–4.93 (0.65–13.39)	0.61 $\pm$ 0.38 (1.68 $\pm$ 1.03)	0.15–1.71 (0.41–4.64)	–0.67 $\pm$ 0.70 (–1.83 $\pm$ 1.92)	–3.7 $\pm$ 3.2	92	2	6	92	8
75+	8	0.87 $\pm$ 0.26 (2.36 $\pm$ 0.72)	0.54–1.34 (1.47–3.64)	0.47 $\pm$ 0.28 (1.28 $\pm$ 0.79)	0.15–0.87 (0.41–2.36)	–0.39 $\pm$ 0.23 (–1.08 $\pm$ 0.63)	–3.8 $\pm$ 2.0	88	12	0	88	12
All	242	1.36 $\pm$ 0.74 (3.69 $\pm$ 2.00)	0.20–4.93 (0.54–13.38)	0.67 $\pm$ 0.38 (1.82 $\pm$ 1.03)	0.15–1.96 (0.41–5.32)	–0.69 $\pm$ 0.55 (–1.87 $\pm$ 1.49)	–4.0 $\pm$ 2.2	95	2.5	2.5	95	5

Mean values are shown $\pm$ s.d.**Table 3** DHEAS change over a 12-year time period in women.

Age group (years)	n	Mean baseline DHEAS ($\mu\text{g/ml}$) ($\mu\text{mol/l}$)	Range of baseline DHEAS ($\mu\text{g/ml}$) ($\mu\text{mol/l}$)	Mean 10- to 14-year follow-up DHEAS ($\mu\text{g/ml}$) ($\mu\text{mol/l}$)	Range of 10- to 14-year follow-up DHEAS ($\mu\text{g/ml}$) ($\mu\text{mol/l}$)	10- to 14-year difference in DHEAS ($\mu\text{g/ml}$) ($\mu\text{mol/l}$)	Annual percentage change DHEAS	Distribution of change by $\pm 10\%$			Distribution of change by regression to the mean criteria	
								Decrease (%)	Same (%)	Increase (%)	Decrease (%)	Increase (%)
60–64	66	0.80 $\pm$ 0.56 (2.20 $\pm$ 1.53)	0.15–3.00 (0.41–8.14)	0.52 $\pm$ 0.34 (1.42 $\pm$ 0.94)	0.15–1.73 (0.41–4.70)	–0.28 $\pm$ 0.40 (–0.77 $\pm$ 1.09)	–2.0 $\pm$ 3.9	71	14	15	82	18
65–69	87	0.79 $\pm$ 0.48 (2.14 $\pm$ 1.30)	0.15–2.52 (0.41–6.84)	0.53 $\pm$ 0.36 (1.46 $\pm$ 0.99)	0.15–1.94 (0.41–5.27)	–0.25 $\pm$ 0.29 (–0.68 $\pm$ 0.81)	–2.1 $\pm$ 4.7	75	12	13	85	15
70–74	46	0.60 $\pm$ 0.40 (1.65 $\pm$ 1.10)	0.15–1.83 (0.41–4.97)	0.41 $\pm$ 0.33 (1.13 $\pm$ 0.92)	0.15–1.65 (0.41–4.48)	–0.19 $\pm$ 0.31 (–0.52 $\pm$ 0.85)	–2.4 $\pm$ 3.1	76	13	11	89	11
75+	8	0.43 $\pm$ 0.29 (1.19 $\pm$ 0.80)	0.15–0.96 (0.41–2.61)	0.32 $\pm$ 0.20 (0.88 $\pm$ 0.57)	0.15–7.40 (0.41–2.01)	–0.11 $\pm$ 0.28 (–0.32 $\pm$ 0.78)	–1.2 $\pm$ 4.3	37.5	37.5	25	88	12
All	207	0.74 $\pm$ 0.49 (2.00 $\pm$ 1.33)	0.15–3.00 (0.41–8.14)	0.50 $\pm$ 0.35 (1.36 $\pm$ 0.95)	0.15–1.94 (0.41–5.27)	–0.24 $\pm$ 0.34 (–0.65 $\pm$ 0.92)	–2.1 $\pm$ 4.1	72	14	14	85	15

Mean values are shown $\pm$ s.d.

Table 4 Determinants of annualized absolute change in DHEAS.

Variables	β coefficient	Model R^2	P value
Baseline DHEAS ($\mu\text{g/ml}$)	-0.054	0.74	<0.0001
Adjusted for age	-0.054	0.74	<0.0001
Adjusted for sex*	-0.052	0.74	<0.0001
Sex*	0.038	0.19	<0.0001
Adjusted for baseline DHEAS	0.006	0.74	0.008
Adjusted for age	0.039	0.21	<0.0001
Age (years)	0.001	0.01	0.008
Adjusted for baseline DHEAS	0.000	0.73	0.734
Adjusted for sex	0.002	0.21	0.004
Weight at baseline (kg)	-0.001	0.04	<0.0001
Adjusted for baseline DHEAS	-0.000	0.74	0.005
Adjusted for age	-0.001	0.05	<0.0001
Adjusted for sex	0.000	0.19	0.314
Weight change (kg)	-0.001	0.00	0.162
BMI at baseline (kg/m^2)	0.000	0.02	0.912
Change in BMI (kg/m^2)	-0.001	0.00	0.371
Smoking	-0.017	0.01	0.008
Adjusted for baseline DHEAS	-0.001	0.74	0.684
Adjusted for age	-0.015	0.02	0.018
Adjusted for sex	-0.017	0.20	0.002

*Male is the reference in all models.

cross-sectional studies (4, 6, 40), and differ from other longitudinal studies that reported increases in DHEAS in 10–40% of ageing individuals of both sexes (7–9, 24, 41). Orentreich *et al.* (7) noted that 13% of 97 normal, healthy men aged 32–83 years showed no change and 20% showed increases in plasma DHEAS over a 13-year period. Kahonen *et al.* (8) reported that DHEAS did not decrease over 5 years in 30% of 71 men and 40% of 200 women aged 75 years and older. Mazat and colleagues (9) reported a 7-year increase in plasma DHEAS in approximately one-third of 119 male, and 171 female, community-dwelling elders whose mean age was 74 years.

We considered several possible explanations for the discrepancies in the findings for DHEAS change among longitudinal studies. First, different definitions

of change were used to classify whether DHEAS levels decreased, increased or stayed the same over time. Kahonen *et al.* (8) chose the standard deviation of mean 5-year changes in DHEAS as a criterion for change. By definition, this imposes set boundaries on the number of individuals who could be classified as staying the same without taking into account baseline levels of DHEAS. Mazat *et al.* (9) used a 19% definition of change for men and women, based on work by Orentreich *et al.*, who reported a mean 19% intra-individual variability in circulating DHEAS levels in four men aged 36–59 over 2 years (6). This definition of change may not be appropriate for women or men older than age 60 (38–39).

Secondly, baseline DHEAS levels are clearly the most important predictor of DHEAS change in both sexes, accounting for 74% of the variability in follow-up DHEAS levels in the present study. Higher initial levels led to more pronounced declines and lower initial levels tended to stay the same or increase. For each 1 $\mu\text{g/ml}$ increment in baseline DHEAS, levels decreased by an additional 0.05 $\mu\text{g/ml}$ per year. The higher proportion of those whose DHEAS levels did not decrease in the Kahonen *et al.* (8) and Mazat *et al.* (9) studies may have been due to the older age and lower initial DHEAS levels at baseline in their samples, as well as to the shorter duration of follow-up, compared with the present study.

Thirdly, no previous studies have reported the effect of regression to the mean in explaining changes in DHEAS over time. Regression to the mean is a common statistical phenomenon that should be considered during the analysis of endocrine studies of repeat hormone measures. The main effect of regression to the mean is to bring extreme measures closer to the population mean on retesting when the measures are imperfectly correlated (10–13), as seen in longitudinal studies of DHEAS. Kahonen *et al.* (8) found correlations of 0.67 between baseline and follow-up DHEAS levels compared with 0.69 in the present study. Moffat and colleagues (24) reported a correlation of 0.80 between baseline and 2-year follow-up DHEAS levels, while Nafziger *et al.* (41) reported a value of 0.83 after a 5-year interval. In the present study, 28% of women had DHEAS levels that remained stable or increased during the study period compared with only 5% of men. When a regression towards the mean artifact was accounted for, only 15% of women showed increasing DHEAS levels. If regression to the mean is not considered, results from longitudinal studies of DHEAS change may be misleading, and the contribution of underlying biological mechanisms to DHEAS change overestimated.

The concept of regression to the mean is a particularly important consideration in longitudinal studies of hormone change when the specimens are obtained from a non-random sample of the population. If subjects or specimens are selected because of a biological

Figure 1 Change in DHEAS levels over a 10- to 14-year period as a function of baseline DHEAS levels among 242 men and 207 postmenopausal women, aged 60–77 years at baseline. Conversion factor: $\mu\text{g/ml} \times 2.74 = \mu\text{mol/l}$.

characteristic that is different from the underlying population average, and if regression to the mean is not properly accounted for, the results of the study may be biased (13). For the present study, only 35% of all baseline participants could be included in the final analysis. According to statistical criteria, survival/participant bias should not have affected our results because age-adjusted DHEAS levels at baseline did not differ among those who were included and those who did not participate in the follow-up study, and DHEAS did not predict mortality in this cohort (42). Nonetheless, our results indicated an overestimation of no change or increase in 13% of the women in this sample when regression to the mean was not accounted for.

The underlying mechanisms of DHEAS change with advancing age are thought to be related to morphological and functional changes in the zona reticularis of the ageing human adrenal cortex. Declines in DHEAS levels have been attributed to atrophy of the adrenal cortical cell mass and a loss of adrenal androgen biosynthesis (43, 44). Observed increases in DHEAS are more difficult to explain. One possibility is that the adrenal gland eventually reaches a minimum secretory threshold, and that the greater frequency of observed increases in DHEAS in older women reflects a fluctuating level of this hormone within a narrow lower range, which may have little physiological importance. Reduced sulphatase activity and decreased renal clearance of DHEAS are other possible mechanisms, but there is a paucity of data to support these explanations.

In the present study, sex was an independent predictor of DHEAS change, with women showing a lower rate of decline and a higher propensity for increasing DHEAS levels. Kahonen *et al.* (8) reported similar sex differences that persisted up to the age of 80 years. A cross-sectional study of the Rancho Bernardo cohort (40) reported that DHEAS levels tended to plateau in women aged 60–80 years, but continued to decline after age 80. Only Mazat *et al.* (9) observed the opposite, with rates of DHEAS decline that were twice as high in older women (3.9%/year) than men (2.3%/year). Our findings suggest that there exist unknown fundamental sex differences in the factors regulating adrenal androgen biosynthesis or clearance. Genetic influences may partially explain the sex-specific variations in DHEAS levels over the lifespan (45–48). Another possibility is that DHEAS plays different physiological roles in men and women, and has correspondingly different feedback mechanisms. Jakubowicz *et al.* (49) showed that weight loss was associated with a 125% increase in serum DHEAS in obese men, but DHEAS levels did not change with weight loss in women after equivalent reductions in BMI and serum insulin. In the present study, DHEAS levels did not vary with changes in BMI in either sex, but changes in BMI were minimal ($-0.3 \pm 2.2 \text{ kg/m}^2$) and few male and female participants were obese (mean baseline BMI 25.6 ± 2.9 and

$24.8 \pm 3.8 \text{ kg/m}^2$ respectively), with the women in this sample being thinner than the national average (50). The sex-specific relationship of DHEAS to body composition and fat distribution warrants investigation.

It should be noted that plasma DHEAS samples from the initial and follow-up visits were not assayed at the same time, and calculated changes in DHEAS levels were based on one single assay at each visit. However, assays were performed by the same person in the same laboratory using the same assay for both visits. Further, single measurements of DHEAS have been shown to characterize average levels reliably in older individuals over a 1- to 3-year period (39, 51). Changes in hormone levels during long-term storage are also unlikely to explain the observed associations. Hormone levels were measured in never previously thawed plasma and levels did not vary by season of sampling or duration of storage. In addition, others have shown that levels of steroid hormones are relatively stable in frozen plasma stored for 3–10 years (7, 52, 53). There was little risk of contamination by over-the-counter DHEA supplement use in this study, because dietary DHEA supplements were not introduced to the public market until the 1990s, years after collection of all plasma samples for the present study.

In summary, this 10- to 14-year longitudinal study of changes in DHEAS levels shows that DHEAS levels continue to decline into late adulthood in nearly all men and in the majority of women. Levels appear to plateau or increase among individuals who have the lowest DHEAS levels, but some of this phenomenon is explained by regression to the mean. Baseline DHEAS appears to be the most important predictor of DHEAS change, with sex being the only other independent predictor. Smoking status, age and baseline weight possibly exert small additional effects on DHEAS change through their effects on baseline DHEAS. Further research is needed to clarify the underlying mechanisms and physiological importance of the significant decrease in circulating DHEAS levels in the majority of older adults, and these investigations need to consider various definitions of change and regression to the mean.

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