**INTRODUCTION**

Dehydroepiandrosterone (DHEA) and its sulphate form (DHEAS) are the most abundant circulating hormones produced by the adrenal glands. An age-associated decrease in DHEA and DHEAS is well established.1 Fetal DHEAS levels are high, but subsequently decline with age, at a rate of about 10% per decade until approximately age 70, when they are only 10% to 20% of their peak value.2 Studies have shown that DHEAS values peak between the second and third decades of life, prior to beginning their decline around 40 years of age.3 Because DHEAS production, and hence concentration, declines over time it has been suggested as a putative biomarker of physiologic aging.4 A clear sex difference in DHEAS concentrations is also seen, with adult women having lower DHEAS concentrations than men.5,6 Levels of DHEAS have been positively associated with mood, energy, and well being,7 with lower DHEAS levels implying poorer psychological well being.8,9 Other studies have shown the decline in DHEAS concentrations over time may be associated with risk of cardiovascular disease (CVD).10,11 Interestingly, sleep quality has been linked to hypothalamic-pituitary-adrenal (HPA) axis function12 and several studies have shown an inverse association between sleep quality and DHEAS.13,14 African Americans experience high levels of stress over their lifetime due to racial discrimination, psychosocial stress and lifestyle behaviors,15,16 but few studies have examined DHEAS in African Americans, in particular as a function of age. We hypothesized that DHEAS would decline with age and be a surrogate for overall stress. Thus, the objectives of our study were to investigate in a sample of African Americans: 1) salivary DHEAS across age groups; 2) associations between DHEAS and measures of biologic health; and 3) association between DHEAS and measures of psychological health.

**METHODS**

**Study Population**

Participants included 170 African Americans men and women between 18 to 60 years of age who were recruited through public advertisements and local churches in the Greater Washington, DC area. The study was approved by the Institutional Review Board of the Uniformed Services University of the Health Sciences, Bethesda, Maryland (MP).
Health Sciences (USUHS), and written informed consent was obtained from all participants. Participants were divided into 3 age groups: young: 18 to 30 \( (n=29) \); middle: 31 to 45 \( (n=50) \) and old: 46 to 60 yrs \( (n=91) \).

**Procedures**

Participants visited the Human Performance Laboratory at USUHS between 0700 and 0900 after an overnight fast and blood pressure and anthropometric evaluations were obtained. Baseline blood samples were obtained for glucose and C-reactive protein assessments. Participants completed psychological questionnaires to assess depression, perceived stress, daily hassles and quality of sleep as described below and collected morning saliva to measure DHEAS.

**Anthropometric Measurements**

Body weight was measured with a calibrated balance beam metric scale to the nearest .1kg and height was measured to the nearest .1cm. Body mass index (BMI) was calculated using height and weight \( (\text{kg/m}^2) \). Percent body fat \( (\text{BF}%) \) was estimated by using bioelectric impedance and calculated with the NHANES III prediction formula. Waist circumference (WC), was measured at the midpoint between the lower rib margin and the iliac crest by using a non-elastic measuring tape. Baseline blood pressure was recorded with a standard blood pressure monitor. Blood pressure measurements were obtained on 2 occasions to ensure accurate assessment and performed with the participant in a supine position. Mean arterial pressure (MAP) was calculated using the formula \( ([2 \times \text{diastolic BP}] + [\text{systolic BP}])/3 \).

**Psychological Assessments**

Self-reported sleep was assessed using the Pittsburgh Sleep Quality Index (PSQI), a 19-item scale which assesses sleep during the past month using seven heterogeneous subscales: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, sleep medication, and daytime dysfunction. The subscales are summed to calculate a global or total sleep quality score, which may range from 0 indicating no difficulty to 21 indicating severe difficulties in all sleep areas. The global score is a well-established sleep quality measure with good internal constancy (Cronbach’s \( \alpha = .83 \)) and equally good test-retest reliability \( (\alpha = .85) \).

Depression was measured using the Beck Depression Inventory (BDI), a 21 item widely used self-report questionnaire and the Center for Epidemiologic Studies Depression Scale (CES-D scale), a short self-report scale designed to measure depressive symptomatology in the general population.

The Daily Hassles Scale (DHS), which consists of 117 items, was used to measure the frequency and severity of a person’s transactions with the environment considered by the person to be stressful events.

Perceived stress was assessed using Cohen’s Perceived Stress Scale (PSS), a 4 item scale used to measure recent exposure to stress. The PSS has adequate internal reliability \( (\alpha = .60) \).

**Physiological and Biochemical Measurements**

Blood glucose concentration was measured with One Touch Ultra monitoring system and high sensitivity C-reactive protein (hsCRP) analyses were carried out with an Immulite 2000 analyzer (Siemens Medical Solutions Diagnostics, Erlanger, Germany); values are expressed in mm/L and mg/L. Participants were instructed to refrain from eating or drinking 60 minutes prior to saliva collections. Saliva samples for measuring DHEAS were collected by the participants upon waking or by 0800 with cotton rolls, which were then placed in vials and stored in their home freezer until ready for shipment. Participants mailed or brought the saliva samples to the investigators, whereupon they were stored at \(-80°C \) until analysis. Salivary DHEAS is stable at room temperature for up to 96 hours prior to any degradation. Saliva samples were quantified for DHEAS by Diagnos-Tech Inc. (Tukwila, Wa, USA) by enzyme linked immunoassay (ELA) procedures; values are expressed in nmol/L.

**Statistical Analysis**

The frequency and distribution of variables were examined and extreme points and normality of distribution were determined. One way analysis of variance (ANOVA) was used to determine differences in variables as a function of sex and age. Scheffe post-hoc tests were used to determine significance across groups. Results are expressed as mean ± SEM and the statistical significance was set at \( P<.05 \). Statistical analyses were performed with SPSS 18.0 for Windows (SPSS Inc., Chicago, Ill., USA).

**Results**

Baseline characteristics and biologic markers are summarized by age in Table 1. All values differed and exhibited significant linear trends by age except for body fat \% and hsCRP, which were comparable across the three age groups. Old and middle age African Americans had significantly higher WC \( (P\leq .001) \), BMI \( (P\leq .05) \), systolic and diastolic blood pressure \( (P\leq .001) \), MAP \( (P\leq .001) \) and fasting blood glucose \( (P\leq .001) \) values compared to the young group. The age-related decline in salivary DHEAS was also significant \( (P\leq .001) \), with the old group having lower salivary DHEAS values \( (14.4± .9) \) compared to the young group \( (25.8±2.4) \). Figure 1 presents the mean scores on self-reported psychological questionnaires by the three age groups. Scores on DHS \( (P\leq .05) \), CES-D \( (P\leq .05) \) and the
Table 1. Baseline characteristics of participants by age and sex, mean ± SEM

<table>
<thead>
<tr>
<th>Variables</th>
<th>18–30 yrs (n=29)</th>
<th>31–45 yrs (n=50)</th>
<th>46–60 yrs (n=91)</th>
<th>Women (n=115)</th>
<th>Men (n=55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morning DHEAS, nmol/L</td>
<td>25.8 ± 2.4^a</td>
<td>21.9 ± 1.9^a</td>
<td>14.4 ± 9.6^b</td>
<td>17.1 ± 1.0</td>
<td>21.6 ± 1.9^c</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>83.9 ± 3.4^a</td>
<td>100.8 ± 2.9^bo</td>
<td>100.0 ± 1.9^bo</td>
<td>97.9 ± 1.9</td>
<td>97.0 ± 2.4</td>
</tr>
<tr>
<td>Body Fat, %</td>
<td>33.9 ± 1.9^a</td>
<td>36.7 ± 1.5^a</td>
<td>36.5 ± 1.1^a</td>
<td>40.9 ± .8</td>
<td>26.3 ± 9^d</td>
</tr>
<tr>
<td>BMI</td>
<td>27.1 ± 1.2^a</td>
<td>32.7 ± 1.3^b</td>
<td>31.7 ± 1.8^c</td>
<td>32.5 ± .8</td>
<td>28.7 ± 9^d</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>121 ± 24^a</td>
<td>133 ± 2.2^bd</td>
<td>139 ± 1.8^bo</td>
<td>133 ± .6</td>
<td>134 ± 2.1</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>74 ± 1.9^a</td>
<td>85 ± 1.6^bo</td>
<td>86 ± 1.3^bo</td>
<td>83 ± 1.1</td>
<td>84 ± 1.9</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>90 ± 1.9^a</td>
<td>101 ± 1.7^bo</td>
<td>104 ± 1.4^bo</td>
<td>100 ± 1.2</td>
<td>101 ± 1.8</td>
</tr>
<tr>
<td>Fasting Glucose, mm/L</td>
<td>5.0 ± .1^a</td>
<td>6.5 ± .3^bo</td>
<td>6.4 ± .2^bo</td>
<td>6.1 ± .1</td>
<td>6.4 ± .3</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
<td>1.9 ± .4^a</td>
<td>4.8 ± 1.0^a</td>
<td>4.2 ± 5.7^a</td>
<td>4.6 ± .6</td>
<td>3.0 ± .7</td>
</tr>
</tbody>
</table>

*Age group means with different superscript letters (a or b) differ significantly.
^P<.05.*
^dP<.01.
^*P<.001.
^* to show significance.

PSQI (P<.001) questionnaires were significantly higher for the old group, relative to the younger group. Overall, significant linear age-dependent trends were noted for all variable except the PSS and BDI.

Baseline characteristics, biologic values and psychological measures were also evaluated as a function of high and low salivary DHEAS. High and low salivary DHEAS values were determined using the lower and upper 25% of the quartile scores. Salivary DHEAS values ranging from lowest to 10.99 nmol/L formed the lower group (n=64) and values ranging from 28 nmol/L to highest formed the higher group (n=31). Those with low DHEAS values had significantly higher WC (P<.05), % body fat (P<.01), BMI (P<.05), systolic blood pressures (P<.01) and mean arterial blood pressures (P<.05). All of these differences disappeared after controlling for age, with the exception of % body fat, which was significantly (P<.01), higher for the low (38.1±1.2) than the high (31.1±1.9) DHEAS groups. Likewise, when psychological measures were evaluated as a function of high and low salivary DHEAS, those with low DHEAS values had significantly higher scores on BDI (P<.05), CESD (P<.01), DHS (P<.05), PSS (P<.01) and PSQI (P<.05) but again, these differences disappeared after controlling for age with the exception of BDI (P<.05), and PSS (P<.05). BDI and PSS scores were significantly higher for those with low DHEAS (BDI: low 6.4±0.8 versus high 3.6±0.9 and PSS: low 4.8±.4 versus high 3.4±.5).

Mean differences in DHEAS were also noted by sex. Baseline characteristics and biologic markers are summarized by sex in Table 1. Salivary DHEAS were significantly (P<.05) higher in African Americans men (21.6±1.9, n=55) than in African Americans women (17.1±1.0, n=115). We also examined the sex differences in age related trends in salivary DHEAS. A significant sex difference (P<.01), was noted for the middle age group with women (n=35; 17.9±1.8) having lower DHEAS concentrations than men (n=15; 31.3±3.8).

**Discussion**

African American men and women have been shown to suffer disproportionately from hypertension, obesity...
and cardiovascular disease and develop these chronic diseases at an earlier age as compared to other ethnic/racial origins.\textsuperscript{20} Moreover, African Americans may experience high levels of stress over their lifetime by virtue of their socioeconomic status, life experiences, and limited social support.\textsuperscript{16,25} Our study shows a clear age-related increase in multiple measures associated with chronic diseases, to include linear increases in % body fat, waist circumference, fasting glucose, and blood pressure. Older African Americans were also more likely to have poor sleep quality, higher scores on measures of depression, and as expected, lower levels of salivary DHEAS than their younger counterparts. The cumulative effect of life stressors and lifestyle behaviors may alter physiologic responses to preclude healthy aging in African Americans.

One important objective of the study was to determine whether declining salivary DHEAS was related to health status in African Americans. Low DHEAS has been associated with many illnesses but the role of salivary DHEAS in health disparities remains unknown.\textsuperscript{26,27} The results of our study indicate a significant age-related decline in DHEAS, which is consistent with well-documented scientific literature.\textsuperscript{28,29} African Americans with low salivary DHEAS concentrations had higher waist circumferences, % body fat, BMI, systolic BP and MAP relative to those with high values of DHEAS. Whereas other studies have shown that DHEA supplementation in men and women improved insulin sensitivity,\textsuperscript{30,31} DHEAS was unrelated to fasting glucose levels.

Previous studies have demonstrated circulating hsCRP is a predictor of cardiovascular disease\textsuperscript{32} and negatively associated with DHEAS levels.\textsuperscript{33} Moreover, some studies comparing hsCRP levels in African Americans to Caucasians suggest African Americans have higher levels when matched for age\textsuperscript{34} whereas others do not.\textsuperscript{35} In our study 67% of the older African Americans group had hsCRP values $>$1mg/L, but no differences were seen across age categories or between high and low salivary DHEAS levels. Thus the relationship between hsCRP and DHEAS in African Americans needs further investigation.

Major depression is often associated with dysregulation of the HPA axis and levels of DHEA and DHEAS are usually affected.\textsuperscript{36,37} High DHEAS levels have also been suggested as a putative marker of resilience and the ability to perform well under stressful conditions.\textsuperscript{38,39} Our results suggest that African Americans with higher DHEAS concentrations had lower scores on depression, daily hassles and perceived stress suggesting that DHEAS may have ameliorative properties in promoting hardness during stress.

Sleep quality, which can influence quality of life and well-being, appears to decline with age; diminished quality of sleep with decreased sleep duration and increased time awake after sleep onset.\textsuperscript{40} Studies in Caucasians have shown significant improvements in well-being, including better sleep and ability to handle stress when taking DHEA supplements.\textsuperscript{41} Our results suggest that lower DHEAS concentrations are associated with poorer sleep quality and various sleep difficulties in African Americans; whether DHEA supplementation would improve sleep quality in African Americans remains to be determined.

Finally, a number of studies have noted a significant age related decline in circulating levels of DHEAS among men and women.\textsuperscript{3,42} Our results confirm that Young African American men and women have significantly higher DHEAS levels than the older group. However, a sex difference was noted within the middle group; women had significantly lower levels than men. This is not unexpected given that major changes in sex hormone metabolism occur earlier in women with advancing age than men.\textsuperscript{4} Whether DHEAS levels in middle age African Americans women could be used as a biological biomarker for health status remains to be determined.

One major strength of our study is the number of participants, a group of 170 African Americans. However several limitations must be noted. First, comparing measures of DHEAS and DHEA in blood and saliva might be important to confirm salivary levels are an appropriate measure in African Americans. Also, only participants for whom complete data were available for the psychological, anthropometric, physiological and biological measurements were included; hence 41 participants were excluded from our initial sample of 211. However, when these two groups (included vs excluded) were compared, no significant differences were noted, with the exception of diastolic blood pressure (included: 84.0±0.9 vs excluded: 79.0±1.8 mm Hg; $P$=.05). Although statistically significant, this may not be physiologically significant. Clearly, longitudinal research with more males will be necessary to determine independent relationships between and among age, DHEAS, and other important health outcomes in both the general population and among African Americans.

In conclusion, DHEAS is related to many health and psychological outcomes including congestive heart failure, vascular risk, anxiety and dysphoric mood, but few studies have looked at DHEAS among African Americans, particularly in a sample of individuals.
of diverse ages. Our study confirmed that DHEAS was related to multiple health and psychological outcomes, but many of these relations were eliminated after controlling for age. The primary remaining outcomes relevant to DHEAS were % body fat, depression and stress. Whether the age-associated decline in salivary DHEAS is a cause or result of obesity, poor sleep and clinically relevant depressive symptoms in older African Americans population is unknown. Its usefulness as a biomarker in sleep, mood and obesity research remains to be determined.

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*Acquisition of funding:* Poth, Deuster

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