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Dehydroepiandrosterone replacement therapy in older adults improves indices of arterial stiffness

Edward P. Weiss,[1,2] Dennis T. Villareal,[1,3] Ali A. Ehsani,[1,4] Luigi Fontana[1,5,6] and John O. Holloszy[1]

[1]Division of Geriatrics and Nutritional Sciences, Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110, USA
[2]Department of Nutrition and Dietetics, Saint Louis University, St. Louis, MO 63104, USA
[3]Division of Geriatrics, University of New Mexico School of Medicine and New Mexico VA Health Care System, Albuquerque, NM 87131, USA
[4]Cardiology Division, Saint Louis Veterans Administration Medical Center – John Cochran Division, Saint Louis, MO 63106, USA
[5]Department of Medicine, Salerno University Medical School, Salerno, Italy
[6]CEINGE Biotecnologie Avanzate, Napoli, Italy

Summary

Serum dehydroepiandrosterone (DHEA) concentrations decrease approximately 80% between ages 25 and 75 year. Aging also results in an increase in arterial stiffness, which is an independent predictor of cardiovascular disease (CVD) risk and mortality. Therefore, it is conceivable that DHEA replacement in older adults could reduce arterial stiffness. We sought to determine whether DHEA replacement therapy in older adults reduces carotid augmentation index (AI) and carotid–femoral pulse wave velocity (PWV) as indices of arterial stiffness. A randomized, double-blind trial was conducted to study the effects of 50 mg day^{-1} DHEA replacement on AI (n = 92) and PWV (n = 51) in women and men aged 65–75 year. Inflammatory cytokines and sex hormones were measured in fasting serum. AI decreased in the DHEA group, but not in the placebo group (difference between groups, −6 ± 2 AI units, P = 0.002). Pulse wave velocity also decreased (difference between groups, −3.5 ± 1.0 m s^{-1}, P = 0.001); however, after adjusting for baseline values, the between-group comparison became nonsignificant (P = 0.20). The reductions in AI and PWV were accompanied by decreases in inflammatory cytokines (tumor necrosis factor α and IL-6, P < 0.05) and correlated with increases in serum DHEAS (r = −0.31 and −0.37, respectively, P < 0.05). The reductions in AI also correlated with free testosterone index (r = −0.23, P = 0.03). In conclusion, DHEA replacement in elderly men and women improves indices of arterial stiffness. Arterial stiffness increases with age and is an independent risk factor for CVD. Therefore, the improvements observed in this study suggest that DHEA replacement might partly reverse arterial aging and reduce CVD risk.

Key words: aging; augmentation index; vasculature.

Correspondence
Edward P. Weiss, Department of Nutrition and Dietetics, Saint Louis University, 3437 Caroline Street, Room 3076, St. Louis, MO 63104, USA; Tel.: +314 977 8523; fax: +314 977 8520; e-mail: eweiss4@slu.edu

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Introduction

Dehydroepiandrosterone (DHEA) and its sulfated form (DHEAS), which will be referred to together as DHEA, are present in a far higher concentration in plasma than any other steroid hormone in humans (Hornsby, 1995). Adrenal production of DHEA begins during puberty and peaks at approximately 20 year. At age approximately 25 year, serum DHEA begins to decline rapidly, so that by age 75 year, DHEA level is approximately 80% lower than at 20 year (Orentreich *et al.*, 1984, 1992). This large decline in DHEA has led to interest in the possibility that development of DHEA deficiency may play a role in the deterioration in physiological and metabolic functions with aging and in the development of aging-related disease processes. In support of this possibility, it has been reported that DHEA level is negatively correlated with mortality and that lower levels of DHEA are associated with a higher risk of developing cardiovascular disease (CVD) in elderly people (Barrett-Connor *et al.*, 1986; Berr *et al.*, 1996; Mazat *et al.*, 2001).

Arterial stiffness increases with advancing age (Vaitkevicius *et al.*, 1993; Hougaku *et al.*, 2006), and elevated central arterial stiffness is a predictor of CVD and all-cause mortality (Laurent *et al.*, 2001; Sutton-Tyrrell *et al.*, 2005; Cohn, 2006). It has been reported that serum DHEA concentration is inversely associated with arterial stiffness (Dockery *et al.*, 2003b; Hougaku *et al.*, 2006; Fukui *et al.*, 2007) and that DHEA has a number of effects that would be expected to prevent and reverse the stiffening of cardiovascular system tissues. These include inhibition of vascular smooth muscle cell proliferation, attenuation of collagen production by cardiac fibroblasts and reduction in left ventricular stiffness, activation of arterial endothelial cell nitric oxide synthase, increase in arterial endothelial cell proliferation and inhibition of arterial endothelial cell apoptosis, and inhibition of vascular inflammation (Liu & Dillon, 2002; Williams *et al.*, 2002, 2004; Iwasaki *et al.*, 2005; Alwardt *et al.*, 2006; Liu *et al.*, 2007; Bonnet *et al.*, 2009). In addition to being a sex hormone precursor, DHEA is an activator of peroxisome proliferator-activated receptor α (PPARα). Dehydroepiandrosterone, therefore, has anti-inflammatory and triglyceride lowering effects (Peters *et al.*, 1996; Poynter & Daynes, 1998; Staels & Fruchart, 2005; LeFebvre *et al.*, 2006). Many of the effects of DHEA are similar to those of the fibrates, which are also PPARα activators (Gizard *et al.*, 2005; Han *et al.*, 2005; Kasai *et al.*, 2006; Ryan *et al.*, 2007; Tziomalos *et al.*, 2009).

While we were conducting a study on the effects of DHEA replacement on glucose tolerance in elderly men and women, a number of the papers, referred to above, were published reporting that DHEA (and fibrates) has effects that would be expected to reduce arterial stiffness. This information stimulated us to add the measurement of carotid artery augmentation index (AI) and carotid–femoral pulse wave velocity (PWV) to our study of the effects of DHEA replacement. In this article, we report the response of these indices of arterial stiffness to 12 months of DHEA replacement on the subgroup of participants on whom arterial stiffness measurements were made.

Methods

Participants

Sedentary, nonsmoking men and women, aged 65–75 year, were recruited from the Saint Louis metropolitan area. Screening tests included

a medical history, physical examination, blood chemistry analysis, hematology, urinalysis, and electrocardiography. Candidates were excluded if they had evidence of chronic infection, a history or evidence of malignancy within the past 5 year (other than innocuous skin cancer), unstable or occult CVD, advanced emphysema, advanced Parkinson's disease, untreated severe hypertension, or diagnosed diabetes. Participants taking medications for dyslipidemia, hypertension, and thyroid dysfunction were required to maintain stable dosing regimens for 6 months prior to enrollment in the study. All participants gave their informed written consent to participate in the study, which was approved by the Human Research Protection Office at Washington University School of Medicine.

Intervention

Participants were randomized to 12 months of 50 mg day^{-1} DHEA or placebo. This DHEA dose was selected because it increases circulating DHEAS levels in older adults to those seen in young adults (Villareal & Holloszy, 2004). All participants received multivitamin and calcium/vitamin D supplements and were advised to maintain their usual dietary and physical activity habits during the study. During monthly meetings with the participants, DHEA or placebo was dispensed, pill counts were performed, and the participants were questioned about adverse events and changes in activity levels, diet, and medications.

Blood pressure and heart rate

Brachial artery blood pressure (BP) was measured (Dinamap 1846SX; Critikon Inc, Tampa, FL, USA) in the left arm after the participant rested quietly for ≥ 5 min in the supine position. Heart rate (HR) was measured by palpation of the radial artery.

Augmentation index

Augmentation index was determined using applanation tonometry (Model #TCB-500; Millar Instruments, Inc., Houston, TX, USA) on the common carotid artery (Laurent *et al.*, 2006). At least 20 digital pulse waves were recorded and analyzed with Windaq software (version 2.31; DATAQ Instruments, Inc., Akron, OH, USA). The software was used to identify the maximum and minimum voltage on each wave form, with the difference corresponding to pulse pressure (PP). The software was also used to generate the second derivative of the pulse wave, which was used for the identification of the 'shoulder' on the upstroke of the raw wave form. The difference between the peak voltage and the voltage at the shoulder was calculated to reflect augmentation pressure (AP). Augmentation index was calculated as AI = 100 × AP/PP for each of the 20 + waveforms, and the resulting values were averaged. To ensure optimal data quality, the technician visually inspected the waveforms to ensure that the landmarks had been properly identified by the software and to omit waveforms that were of suboptimal quality owing to artifacts or irregular heartbeats. When analyses were questionable (e.g. large variation in AI values among waveforms), a second (blinded) technician re-analyzed the waveforms; when discrepancies between technicians occurred, the analyses were reviewed by both technicians together, and if the differences could not be remediated, the data were excluded from the analyses for this report.

Pulse wave velocity

Pulse wave velocity was determined by transcutaneous Doppler flow measurements (Model 806-CB; Parks Medical Electronics, Inc., Aloha, OR, USA) at the right common carotid artery and the right femoral artery (Laurent *et al.*, 2006). Twenty Doppler wave forms were recorded (Windaq software, version 2.31; DATAQ Instruments, Inc.) at the two sites simultaneously. Pulse transit time was determined as the difference in pulse arrival times for the carotid and femoral sites and was based on foot-to-foot comparisons of wave forms from the two sites, with the foot being identified as the peak on the second derivative of the pulse wave. The distances between the aorta and the carotid site and the aorta and the femoral site were measured over the skin using the second intercostal space as a landmark for the aorta; the difference between these distances was considered propagation distance (Karamanoglu, 2003). Pulse wave velocity for each carotid–femoral pair of waveforms was calculated as propagation distance in meters divided by transit time in seconds. The average of the 20 waveforms was used to reflect the PWV for one test. Quality control procedures were identical to those described above for the AI method.

Blood analyses

In the morning after an overnight fast, blood was collected from an arm vein; serum was isolated using centrifugation. The serum samples were stored at −20 °C for later batch analyses. Commercially available ELISA assay kits (Quantikine High Sensitive; R&D Systems, Minneapolis, MN, USA) were used to quantify serum concentrations of interleukin-6 (IL-6) and tumor necrosis factor α (TNFα). Sex hormone–binding globulin (SHBG), total testosterone, and DHEAS were measured using chemiluminescent assays (Immulite 2000; Diagnostic Products Corporation, San Diego, CA, USA); total estradiol was measured using an ultrasensitive radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX, USA). Free testosterone index was calculated as total testosterone/SHBG, where the units are nM for testosterone and nM for SHBG. Free estradiol index was calculated as (total estradiol/1000)/SHBG, where the units are pM for estradiol and nM for SHBG. White blood cells, lymphocytes, and lipids were measured in plasma by the medical center's clinical laboratory improvement amendments (CLIA)-certified clinical laboratory.

Height and weight

Body weight and height were measured in the morning, after an overnight fast, while the participant was wearing only underwear and a hospital gown. Body mass index (BMI) was calculated (kg m^{-2}).

Physical activity and energy intake

Habitual physical activity levels were evaluated using a questionnaire that focuses on habitual exercise and nonexercise physical activity performed during the prior 3 months (The aerobics center longitudinal study physical activity questionnaire, 1997). Energy intake was evaluated by having the participants record 4-day food diaries, which were analyzed by the study dietitian using computerized nutrient analysis (Nutrition Data System for Research, versions 4.05, 4.06, and 5.0; Nutrition Coordination Center, University of Minnesota, Minneapolis, MN, USA). Prior to the diary recording period, participants received detailed instructions from the dietitian on how to measure and record all foods, beverages, and supplements consumed. After the recording period, the dietitian reviewed the diary and queried the participant, as needed to clarify any incomplete or ambiguous entries in the diary.

Statistical analyses

Comparisons of baseline characteristics between the DHEA and placebo groups were performed using independent *t*-tests, chi-square tests, and Fisher's exact tests. Outcomes were analyzed with analysis of covariance (ANCOVA), in which the independent variable was study group, the dependent variable was the change in the outcome (i.e. final value minus baseline value), and the covariate was the baseline value of the outcome. Additional ANCOVAS were performed in which a sex by study group interaction term was included to evaluate the equality of responses to DHEA in men and women. Paired *t*-tests were used for within-group comparisons of baseline and 1-year data. Spearman correlations were performed on data from both groups combined and used to identify associations between variables. Data are presented as arithmetic means ± SE unless noted otherwise. Significance was accepted at $P \leq 0.05$, and all tests were two-tailed. Analyses were conducted with SAS for Windows XP Pro (version 9.2; SAS Institute, Cary, NC, USA).

Results

Participants

Among the 659 volunteers who inquired about the study, 335 did not meet the inclusion criteria and 188 were not interested in participating. The remaining 136 subjects were enrolled and randomized; among these, seven dropped out before completing the study (Fig. 1). As mentioned above, the AI and PWV measures were phased-in, after the larger study had begun. Therefore, data are not available for all participants. Additionally, some data are missing because of the presence of a carotid bruit (contraindication for the vascular examination) or because the acquired pulse wave forms were not suitable for analysis. Therefore, the sample size for the present report was 92 subjects ($n = 46$ for each group; Fig. 1), except for PWV, for which the sample was 51 subjects (DHEA, $n = 27$; placebo, $n = 24$).

On average, the participants were 70 years of age with BMI in the overweight range (Table 1). The demographic and baseline characteristics did not differ between groups. The percentage of participants who were taking medications or vitamin supplements that could affect the study outcomes was similar between groups. Furthermore, the percentage of participants with prior diagnoses of hypertension or CVD was similar in the two groups.

Table 1 Subject characteristics

	DHEA ($n = 46$)	Placebo ($n = 46$)	Between-group *P* value
Sex			
Men	20 (43%)	22 (48%)	0.68
Women	26 (57%)	24 (52%)	
Race			
African American/Black	1 (2%)	2 (4%)	0.56
White	45 (98%)	44 (96%)	
Education			
< College degree	24 (52%)	19 (41%)	0.52
College degree	12 (26%)	13 (28%)	
Graduate School	10 (22%)	14 (30%)	
Age, yr	70 ± 3	70 ± 3	0.95
Weight, kg			
Men	87.9 ± 14.6	83.4 ± 12.6	0.29
Women	71.4 ± 16.6	76.1 ± 18.9	0.36
BMI, kg m^{-2}	27.7 ± 5.5	27.8 ± 5.3	0.95
Medication use			
Anti-dyslipidemic	16 (35%)	24 (52%)	0.09
Antihypertensive	23 (50%)	23 (50%)	1.00
Multivitamin	28 (61%)	33 (72%)	0.27
Vitamin C and/or E	23 (50%)	18 (39%)	0.29
Self-reported diagnoses			
Cardiovascular disease	5 (10%)	6 (13%)	0.75
Hypertension	20 (43%)	18 (39%)	0.67

BMI, body mass index; DHEA, dehydroepiandrosterone.
Values are means ± SD or *n* (% of participants). *P*-values are for independent *t*-tests for quantitative data and chi-square tests or Fisher's exact tests for counts.

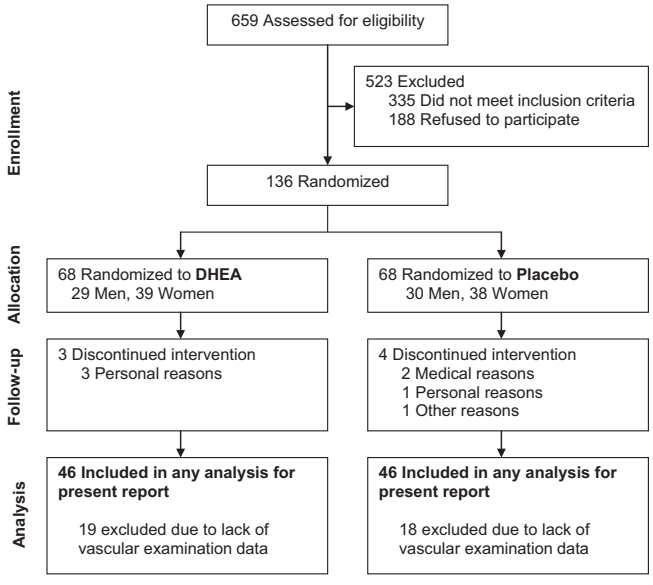

Fig. 1 Consort diagram indicating sample sizes at each stage during the study. DHEA, dehydroepiandrosterone.

Compliance and safety

Pill compliance has been reported previously (Weiss *et al.*, 2009) and was 94.4 ± 0.4% in the DHEA group and 95.6 ± 0.4% in the placebo group. Circulating DHEAS increased from 59 ± 5 to 333 ± 20 μg dL^{-1} ($P < 0.0001$) in the DHEA group and did not change in the placebo group (baseline: 56 ± 8 μg dL^{-1}, 1 year: 46 ± 5 μg dL^{-1}; $P = 0.09$; $P < 0.0001$ vs. DHEA group).

As reported in greater detail previously for a larger sample ($n = 136$; Weiss *et al.*, 2009), a total of 12 serious adverse events and 124 minor side effects were documented; the frequencies of these did not differ between groups. Serum prostate specific antigen concentrations in men did not change in either group during intervention, nor were there differences between groups. Based on mammograms and pap smears, no breast cancer or cervical abnormalities were identified in women.

Augmentation index

Augmentation index decreased in the DHEA group and tended to increase in the placebo group (Fig. 2). These findings were not affected by the inclusion of BP or heart rate as covariates. Likewise, adjustment of the AI data to a standardized heart rate of 75 beats per min (based on the inverse relationship between AI and HR of 4.8 AI units per 10 beats

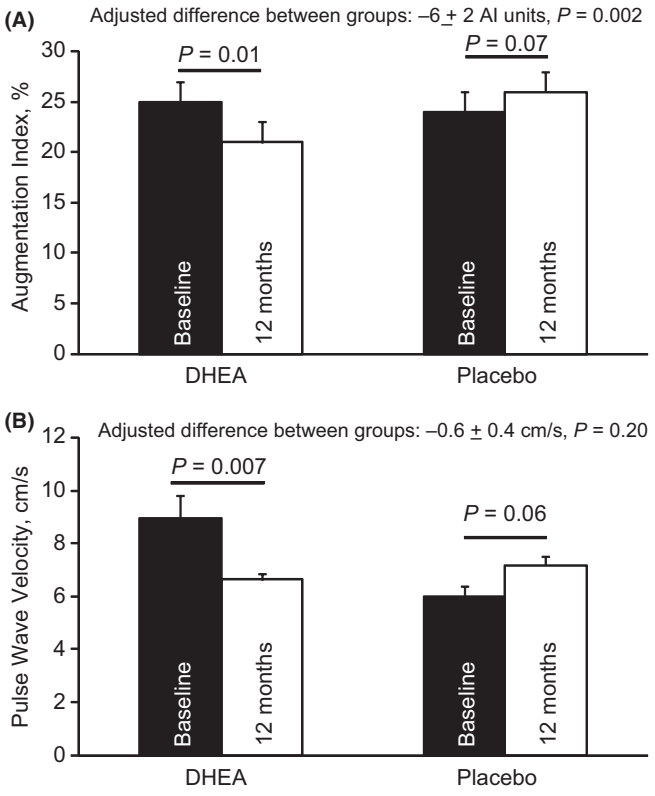

Fig. 2 Changes in augmentation index (AI, panel A) and pulse wave velocity (PWV, panel B) in response to 12 months of 50 mg day^{-1} dehydroepiandrosterone (DHEA) supplementation or placebo. Adjusted differences reflect the comparison of the changes in the DHEA and placebo groups after adjusting for baseline values. Sample size for the AI data is $n = 46$ in each group. For the pulse wave velocity (PWV) data, the sample size was $n = 27$ for DHEA and $n = 24$ for placebo groups. The unadjusted between-group comparison of PWV results was significant ($P = 0.001$).

per min; Wilkinson *et al.*, 2000, 2002) did not alter the significance of the results. Exclusion of ten participants in the DHEA group and nine participants in the placebo group who started, stopped, or had dose changes in BP medications during the intervention did not change the statistical significance of the results (between-group comparison, $P = 0.004$). The DHEA-associated improvements in AI did not differ ($P = 0.58$) between men (–7.4 ± 2.8 AI units, $P = 0.01$) and women (–5.3 ± 2.6 AI units, $P = 0.05$).

Pulse wave velocity

Pulse wave velocity decreased in the DHEA group and tended to increase in the placebo group (Fig. 2), resulting in a significant difference between groups (–3.5 ± 1.0 m s^{-1}, $P = 0.001$). However, after accounting for a substantial difference in baseline values between groups, the between-group comparison became a weak, nonsignificant ($P = 0.20$) trend. These findings were not affected by the inclusion of BP or heart rate as covariates (data not shown). Furthermore, the results were not affected by exclusion of eight participants in the DHEA group and six participants in the placebo group who started, stopped, or had dose changes in BP medications during the intervention. The responses for men (–0.9 ± 0.6 m s^{-1}, DHEA vs. placebo, $P = 0.14$) and women (–0.3 ± 0.6 m s^{-1}, $P = 0.59$) did not differ significantly ($P = 0.44$).

Body mass index and body weight

There were tendencies for reductions in BMI and body weight with DHEA supplementation (Table 2). As reported in a previous paper from this study (Weiss *et al.*, 2011), these effects appear to be specific to men (differences between the DHEA and placebo groups: BMI, –0.9 ± 0.3 kg m^{-2}, $P = 0.002$; body weight: –2.3 ± 0.7 kg, $P = 0.003$), with no effect seen in women (BMI, 0.0 ± 0.3 kg m^{-2}, $P = 0.99$; body weight: 0.0 ± 0.7 kg, $P = 0.96$; group by sex interaction, both $P < 0.05$).

Blood pressure and heart rate

The DHEA and placebo groups did not differ with respect to the 1-year changes in supine resting systolic or diastolic BP, or resting heart rate (Table 2). However, as compared to men, women tended to have more favorable responses to DHEA replacement, with respect to improvements in systolic BP (women: –7 ± 3 mmHg; men: 5 ± 4 mmHg; $P = 0.02$ for men vs. women), diastolic BP (women: –2 ± 2 mmHg; men, 4 ± 2 mmHg; $P = 0.06$ for men vs. women), and resting HR (women: –4 ± 1 beats min^{-1}; men, 0 ± 2 beats min^{-1}; $P = 0.09$ for men vs. women).

Results from seated bilateral brachial pressures measured in triplicate were available on a subset of study participants ($n = 20$ in each group). As was the case for supine BPs, there was no difference in BP changes between the DHEA and placebo groups. However, the differences observed between men and women, with respect to DHEA-induced changes in systolic and diastolic BP, became nonsignificant ($P = 0.44$ and $P = 0.14$, respectively).

Plasma lipids

As reported previously (Weiss *et al.*, 2011), DHEA replacement resulted in lower plasma triglyceride concentrations and did not alter total or LDL cholesterol levels (Table 2). Furthermore, although DHEA did not alter HDL cholesterol levels in the group as a whole (Table 2), women experienced a reduction in HDL cholesterol (DHEA: –6 + 2 mg dL^{-1}, placebo, 4 ± 2 mg dL^{-1}, $P = 0.0007$ DHEA vs. placebo).

Physical activity levels and dietary energy intake

There was no difference between the DHEA and placebo groups with respect to changes in habitual physical activity levels or dietary energy intake (Table 2).

Sex hormones

Sex hormone data have been presented previously for a larger group of subjects from this trial (Weiss *et al.*, 2009) and are being presented here to assist in the interpretation of AI and PWV data. For both men and women, serum testosterone concentrations increased in the DHEA group, but not in the placebo group. However, the between-group comparison of these responses was only significant for women (Table 3). Free testosterone index, estradiol, and free estradiol index increased in men and women in the DHEA group, but not in the placebo group; the between-group comparisons of responses were all significant for men and women (Table 3).

Inflammatory markers and white blood cells

As compared to the placebo group, the DHEA group had favorable changes in serum TNFα and IL-6 concentrations (Table 4). The effect on

Table 2 Effect of 12 months of dehydroepiandrosterone (DHEA) supplementation or placebo on cardiovascular disease risk factors, physical activity levels, and energy intake

	DHEA	Placebo	Adjusted difference between groups	Between group *P* value
BMI, kg m^{-2}				
Baseline	27.7 ± 0.8	27.8 ± 0.8	−0.4 ± 0.2	0.07
12 months	27.8 ± 0.8	28.3 ± 0.8		
Change	0.1 ± 0.1	0.5 ± 0.1		
Within-group *P* value	0.58	0.002		
Body weight, kg				
Baseline	78.6 ± 2.6	79.6 ± 2.4	−1.0 ± 0.5	0.06
12 months	78.5 ± 2.5	80.5 ± 2.5		
Change	−0.1 ± 0.4	0.9 ± 0.4		
Within-group *P* value	0.84	0.02		
Systolic BP, mmHg				
Baseline	126 ± 2	132 ± 3	0 ± 2	0.89
12 months	128 ± 2	132 ± 2		
Change	1 ± 2	0 ± 2		
Within-group *P* value	0.53	0.79		
Diastolic BP, mmHg				
Baseline	68 ± 1	71 ± 1	0 ± 1	0.81
12 months	70 ± 1	72 ± 1		
Change	2 ± 1	1 ± 1		
Within-group *P* value	0.06	0.25		
Heart rate, beats min^{-1}				
Baseline	64 ± 1	63 ± 1	−3 ± 2	0.10
12 months	64 ± 1	66 ± 1		
Change	0 ± 1	3 ± 1		
Within-group *P* value	0.76	0.03		
Triglycerides, mg dL^{-1}				
Baseline	112 ± 10	102 ± 9	−15 ± 6	0.03
12 months	99 ± 9	105 ± 10		
Change	−14 ± 5	3 ± 5		
Within-group *P* value	0.006	0.56		
Total cholesterol, mg dL^{-1}				
Baseline	189 ± 5	184 ± 6	−5 ± 5	0.33
12 months	180 ± 5	182 ± 7		
Change	−9 ± 4	−3 ± 4		
Within-group *P* value	0.02	0.54		
LDL cholesterol, mg dL^{-1}				
Baseline	109 ± 5	103 ± 6	−3 ± 4	0.54
12 months	104 ± 5	101 ± 7		
Change	−4 ± 3	−2 ± 3		
Within-group *P* value	0.13	0.15		
HDL cholesterol, mg dL^{-1}				
Baseline	58 ± 3	58 ± 3	−3 ± 2	0.19
12 months	56 ± 3	59 ± 4		
Change	−2 ± 2	1 ± 2		
Within-group *P* value	0.34	0.37		
Physical Activity, kcal day^{-1}				
Baseline	453 ± 46	511 ± 58	68 ± 50	0.18
12 months	459 ± 55	431 ± 45		
Change	−2 ± 35	−71 ± 36		
Within-group *P* value	0.95	0.05		
Energy intake, kcal day^{-1}				
Baseline	2226 ± 66	2183 ± 79	−133 ± 72	0.07
12 months	2093 ± 66	2190 ± 81		
Change	−40 ± 50	94 ± 50		
Within-group *P* value	0.43	0.07		

Body mass, plasma lipid, and energy intake data have been reported previously for a larger sample (Weiss *et al.*, 2011). Values are arithmetic means ± SE except for mean differences between groups, which have been adjusted for baseline values. Between-group *P* values reflect the between-group comparison change-scores from ANCOVAS that included baseline values as the covariate. Within-group *P* values are from paired *t*-tests. Lipid data do not include participants who had changes in lipid medications during the study.

Table 3 Circulating hormones in response to 12 months of dehydroepiandrosterone (DHEA) supplementation or placebo

	Men				Women			
			Between group				Between group	
	DHEA (n = 20)	Placebo (n = 22)	Difference	P	DHEA (n = 26)	Placebo (n = 24)	Difference	P
Tot. testosterone, ng dL^{-1}								
Baseline	426 ± 30	426 ± 38	43 ± 29	0.15	24 ± 1	22 ± 1	28 ± 5	< 0.0001
12 months	496 ± 32	453 ± 32			54 ± 5	24 ± 2		
Change	70 ± 19	28 ± 25			30 ± 4	2 ± 2		
Within-group P value	0.001	0.28			< 0.0001	0.27		
Free testosterone index × 10^{-2}								
Baseline	43.9 ± 2.6	40.7 ± 3.0	12.5 ± 3.0	0.0002	2.2 ± 0.2	2.2 ± 0.2	3.3 ± 0.6	< 0.0001
12 months	54.6 ± 3.3	39.7 ± 2.6			5.7 ± 0.6	2.4 ± 0.3		
Change	10.7 ± 2.4	−1.0 ± 2.0			3.5 ± 0.5	0.2 ± 0.2		
Within-group P value	0.0003	0.63			< 0.0001	0.27		
Total estradiol, pg mL^{-1}								
Baseline	18.5 ± 1.3	15.9 ± 0.9	6.3 ± 1.3	< 0.0001	10.0 ± 1.0	11.3 ± 0.8	6.3 ± 0.9	< 0.0001
12 months	22.2 ± 1.2	14.6 ± 0.8			16.2 ± 0.8	10.4 ± 0.6		
Change	3.7 ± 1.2	−1.3 ± 0.8			6.2 ± 1.1	−0.9 ± 0.5		
Within-group P value	0.008	0.14			< 0.0001	0.08		
Free estradiol index × 10^{-4}								
Baseline	21.7 ± 2.2	17.7 ± 1.6	9.3 ± 1.9	< 0.0001	10.0 ± 1.3	13.0 ± 1.8	10.1 ± 1.8	< 0.0001
12 months	27.2 ± 2.4	15.0 ± 1.2			19.0 ± 2.1	11.5 ± 1.5		
Change	5.5 ± 1.7	−2.7 ± 1.0			9.0 ± 1.6	−1.5 ± 0.7		
Within-group P value	0.004	0.02			< 0.0001	0.04		
SHBG, nM								
Baseline	34.4 ± 2.1	37.0 ± 2.7	−4.2 ± 1.5	0.007	42.3 ± 2.6	40.3 ± 3.2	−8.3 ± 1.9	< 0.0001
12 months	32.9 ± 2.6	39.7 ± 2.8			35.9 ± 2.4	42.5 ± 3.6		
Change	−1.5 ± 1.0	2.8 ± 1.0			−6.4 ± 1.4	2.1 ± 1.3		
Within-group P value	0.15	0.01			0.0001	0.11		

Values are arithmetic means ± SE except for mean differences between groups which have been adjusted for baseline values. Between-group P values reflect the between-group comparison change-scores from ANCOVAs that included baseline values as the covariate. Within-group P values are from paired t-tests. SHBG, sex hormone–binding globulin. To convert testosterone to SI units (nM), divide by 28.82; to convert estradiol to SI units (pM), divide by 0.27.

Table 4 Circulating inflammatory markers and white blood cells in response to 12 months of dehydroepiandrosterone (DHEA) supplementation or placebo

	DHEA	Placebo	Adjusted difference between groups	Between-group P value
TNFα, pg mL^{-1}				
Baseline	1.45 ± 0.15	1.22 ± 0.15	−0.56 ± 0.23	0.02
12 months	1.21 ± 0.10	1.61 ± 0.26		
Change	−0.24 ± 0.09	0.38 ± 0.22		
Within-group P value	0.02	0.09		
IL-6, pg mL^{-1}				
Baseline	2.61 ± 0.24	2.41 ± 0.17	−0.80 ± 0.20	0.0001
12 months	2.21 ± 0.16	2.90 ± 0.20		
Change	−0.40 ± 0.15	0.49 ± 0.19		
Within-group P value	0.01	0.01		
WBC, k $cumm^{-1}$				
Baseline	5.7 ± 0.2	5.3 ± 0.2	−0.1 ± 0.2	0.77
12 months	5.8 ± 0.2	5.6 ± 0.2		
Change	0.1 ± 0.2	0.3 ± 0.1		
Within-group P value	0.65	0.04		
Lymphocytes, k $cumm^{-1}$				
Baseline	1.55 ± 0.07	1.48 ± 0.05	−0.07 ± 0.06	0.20
12 months	1.57 ± 0.07	1.58 ± 0.06		
Change	0.02 ± 0.04	0.11 ± 0.04		
Within-group P value	0.67	0.02		

TNFα, tumor necrosis factor α; IL-6, interleukin-6; WBC, white blood cells.
Values are arithmetic means ± SE except for mean differences between groups which have been adjusted for baseline values. Between-group P values reflect the between-group comparison change-scores from ANCOVAs that included baseline values as the covariate. Within-group P values are from paired t-tests.

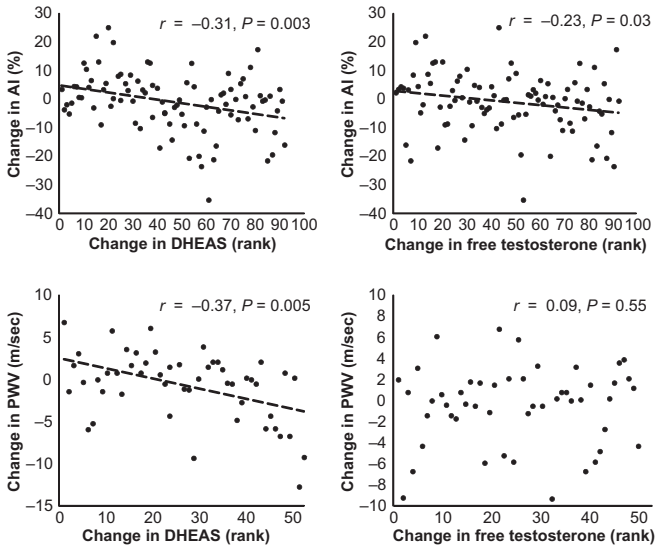

Fig. 3 Associations between changes in indices of arterial stiffness and changes in serum DHEAS concentrations and free testosterone index. Correlation analyses were performed by using Spearman rank correlations because the data were not normally distributed. DHEAS and free testosterone index data were rank-transformed for the figure with high ranks corresponding to large increases in dehydroepiandrosterone (DHEA) or free testosterone index.

TNFα was attributed to a modest decrease in TNFα in the DHEA group, while TNFα tended to increase in the placebo group. The effect on IL-6 was attributed to a significant decrease in the DHEA group, while the placebo group experienced a significant increase. Tumor necrosis factor and IL-6 data from this study have been reported previously (Weiss *et al.*, 2011), but are reported here for this subgroup of participants because of their importance in vascular health. There were no differences between groups for white blood cell or lymphocyte counts (Table 4). There were no differences between men and women, with respect to the DHEA-induced changes in inflammatory markers or white blood cells (all $P > 0.48$ for sex by group interactions).

Correlations

Decreases in AI were correlated with increases in serum DHEAS ($r = -0.31$, $P = 0.003$) and increases in total ($r = -0.26$, $P = 0.01$) and free testosterone index ($r = -0.23$, $P = 0.03$) (Fig. 3). Reductions in AI also tended to correlate with reductions in SHBG ($r = 0.18$, $P = 0.08$), IL-6 ($r = 0.21$, $P = 0.06$), and TNFα ($r = 0.19$, $P = 0.07$). Changes in AI were not correlated with changes in estradiol or free estradiol index, BMI or body weight, plasma lipids, or dietary energy intake or habitual physical activity levels ($r = -0.07$–0.18, $P > 0.05$).

Decreases in PWV were correlated with increases in serum DHEAS concentrations ($r = -0.37$, $P = 0.005$) (Fig. 3) and tended to correlate with decreases in SHBG ($r = 0.25$, $P = 0.07$) and decreases in triglycerides ($r = 0.25$, $P = 0.09$; excludes subjects who had changes in lipid medications), but were not associated with changes in other lipids or with changes in sex hormones, inflammatory cytokines, body mass or BMI, energy intake, or physical activity ($r = -0.19$–0.18, $P > 0.05$).

Discussion

The purpose of this study was to evaluate the hypothesis that DHEA replacement improves arterial elasticity in elderly men and women. This hypothesis was based on evidence that DHEA, like fibrates (Tziomalos *et al.*, 2009), is a PPARα activator (Peters *et al.*, 1996; Tamasi *et al.*, 2008) and mediates a number of adaptations, similar to those induced by fibrates (Williams *et al.*, 2002; Liu *et al.*, 2007), that would be expected to improve cardiovascular elasticity. Our finding that DHEA replacement reduced AI and PWV in elderly men and women supports this hypothesis. Altman *et al.* (2008) have reported that inhibition of PPARα prevents an anti-inflammatory effect of DHEA on human aortic endothelial cells. This finding and our observation of decreases in IL-6 and in plasma triglyceride levels (Weiss *et al.*, 2011) are in keeping with the possibility that the effects of DHEA observed in this study were at least partially mediated by activation of PPARα.

An additional mechanism that may have contributed to the decrease in arterial stiffness in response to DHEA replacement is the increase in free testosterone. This increase, which was modest in the men but large, in relative terms, in the women, correlated with the reduction in AI. Physiological levels of testosterone appear to be beneficial for vascular health, as evidenced by the finding that patients undergoing androgen suppression therapy for prostate cancer and hypogonandal men have greater arterial stiffness than age-matched controls (Dockery *et al.*, 2003a). Testosterone replacement reverses this increase in arterial stiffness (Yaron *et al.*, 2009).

The 24% decrease in AI that we observed with DHEA supplementation is large in the context of arterial aging. Based on a 0.30 percentage point per year increase in AI during adulthood (Vaitkevicius *et al.*, 1993), the DHEA-induced reduction in AI is equivalent in magnitude to a 20-year reversal of arterial aging. Furthermore, based on a meta-analysis that shows that every ten percentage point increase in AI corresponds with a approximately 36% greater risk for cardiovascular and all-cause mortality during a 4-year follow-up period (Vlachopoulos *et al.*, 2010a), the reductions in AI that we observed would be expected to correspond with a 17% reduction in mortality risk (Vlachopoulos *et al.*, 2010b).

To our knowledge, no other studies have evaluated the effects of DHEA supplementation on indices of arterial stiffness in older adults. However, in middle-aged patients with low levels of DHEA owing to primary or secondary adrenal insufficiency, it has been reported that DHEA supplementation does not alter AI or aortic PWV (Rice *et al.*, 2009). It is possible that the shorter-term, 12-week supplementation period did not allow sufficient time for the vascular remodeling that may be important for reductions in arterial stiffness. Furthermore, the pathogenesis and secondary effects of adrenal insufficiency are distinct from the hormonal changes that occur during normal aging; therefore, conditions present in patients with adrenal insufficiency may have precluded DHEA-related changes in arterial stiffness.

The subject sample used in this study was heterogeneous with respect to medical history, medications, and sex, which might be viewed as a limitation. Some participants had diagnosed CVD (12%), were taking anti-hypertensive medications (52%), or were taking anti-dyslipidemic medications (approximately 43%). Although our study was not powered to perform subanalyses after excluding subjects with CVD or those on BP or lipid medications, exclusion of each of these subgroups did not alter the significance of the results (data not shown), despite the smaller sample sizes for these analyses. Furthermore, the responses to DHEA replacement therapy in men and women did not differ; however, this finding should be interpreted cautiously, as the sample sizes were not optimal for testing hypotheses about sex differences. Taken together, these subanalyses and sex comparisons indicate that our findings are robust and can be generalized to a fairly homogeneous population of elderly men and women.

While the results from the PWV data support the hypothesis that DHEA supplementation reduces arterial stiffness, this finding has limitations. First, because PWV measures were added later in the study, the sample size was small (approximately half of that for AI), thereby resulting in less statistical power. Additionally, by chance, there was a large baseline difference in PWV between the DHEA and placebo groups, thereby making the interpretation of results difficult. Nonetheless, there was a clear significant improvement in PWV in the DHEA group, and this improvement was statistically greater than the change observed in the placebo group ($P = 0.001$). Only after accounting for the baseline differences in baseline values did the between-group statistical test become nonsignificant ($P = 0.20$).

In conclusion, DHEA replacement in elderly men and women reduces arterial stiffness. Arterial stiffness increases with age and is an independent risk factor for CVD. Therefore, the improvement in arterial elasticity suggests that DHEA replacement might partly reverse arterial aging and reduce CVD risk in older men and women.

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