

ORIGINAL ARTICLE

Dehydroepiandrosterone substitution in female adrenal failure: no impact on endothelial function and cardiovascular parameters despite normalization of androgen status

Jens Juel Christiansen*, Niels Holmark Andersen*, Keld E. Sørensen†, Erik Morre Pedersen‡, Paul Bennett§, Marianne Andersen¶, Jens Sandahl Christiansen*, Jens Otto Lunde Jørgensen* and Claus Højbjerg Gravholt*

*Medical Department M, Aarhus Sygehus, Aarhus University Hospital, Denmark, †Department of Cardiology, Skejby Sygehus, Aarhus University Hospital, ‡The MR Centre, Skejby Sygehus, Aarhus University Hospital, Denmark, §Statens Serum Institute, Copenhagen, Denmark and ¶Department of Endocrinology, Odense University Hospital, Odense, Denmark

Summary

Background Female adrenal insufficiency implicates reduced production of the adrenal androgen precursor dehydroepiandrosterone (DHEA) and low androgen levels. Oral DHEA restores androgen deficit but the clinical implications and safety of substitution therapy is uncertain. A putative DHEA receptor in vascular endothelium has been described and *in vitro* studies have shown involvement of DHEA in NO dependent pathways.

Aim To evaluate effects of DHEA substitution on cardiovascular parameters.

Design Six months randomized, double-blind, placebo-controlled crossover study. Treatment consisted of DHEA 50-mg or placebo. Each treatment period was followed by a 2-month washout period. **Material and methods** Ten females with documented adrenal failure were included. Androgen levels were measured. Cardiovascular evaluation was performed before and after every treatment period. Two patients left the study because of skin side effects and anxiety, respectively. All patients had low circulating androgens baseline and normal range androgens during DHEA treatment. We examined patients with noninvasive endothelial cell function, magnetic resonance imaging (MRI)-based cardiac output, echocardiography, ambulatory 24-h blood pressure and maximal oxygen consumption.

Results DHEA treatment normalized androgen status to levels seen in healthy women. DHEA and placebo treatment had no effect on echocardiographic parameters of myocardial dimensions or systolic and diastolic function, noninvasive endothelial cell function at the level of the brachial artery, 24-h blood pressure and heart rate, cardiac output and maximal oxygen consumption during exercise

cycle testing. Remarkably, all participants had evidence of concentric left ventricular remodelling by echocardiography.

Conclusion Restoration of physiological androgen levels using 6 months of DHEA replacement in this pilot study did not affect cardiovascular parameters and endothelial function in female adrenal insufficiency.

(Received 5 September 2006; returned for revision 26 September 2006; finally revised 11 October 2006; accepted 15 October 2006)

Introduction

Dehydroepiandrosterone (DHEA) and its sulphate (DHEAS) are secreted from the adrenals as the most abundant circulating steroid, primarily in response to ACTH. DHEA dissociates from cortisol concentrations, which remain stable during a life span, with peak production from adolescence to early adulthood and a steady decline with age.¹

Regulation and physiological function of DHEA are poorly understood. DHEA is a sex hormone precursor contributing to circulating concentrations of androgens and oestrogens^{2–7} through conversion in peripheral tissues.¹ DHEA may be a discriminator of morbidity during ageing, at least in primates.⁸ DHEAS levels are decreased in chronic heart failure in proportion to severity.⁹ DHEA is also depressed during hyperinsulinaemic states and is proposed to be a link to atherosclerosis.¹⁰

DHEA has been reported to activate endothelial cell nitric oxide (NO) synthase by a specific membrane-bound G-protein coupled receptor in bovine endothelial cells¹¹ and to inhibit human vascular smooth muscle cell growth via a putative receptor and not through the androgen or oestrogen receptor¹² suggesting that DHEA and/or DHEAS may modulate endothelial function.

The secretory failure of the adrenal glands due to local or pituitary disease is followed by hypocortisolism and reduced levels of androgen precursors. Female patients are characterized by relative androgen deficiency not seen in their male counterparts.

Correspondence: Claus Højbjerg Gravholt, MD, PhD, Medical Department M (Endocrinology and Diabetes), Århus Sygehus NGB, DK-8000 Aarhus C, Denmark. Tel.: +45 89 49 20 71; Fax: +45 89 49 20 10; E-mail: ch.gravholt@dadlnet.dk

DHEA substitution therapy restores the biochemical deficit with possible effects on quality of life parameters.^{3,4,7} Here, we have examined the effect of DHEA on endothelial and cardiac function, in a double-blind, placebo-controlled 6 months crossover study. We hypothesized that supplementation with DHEA would improve vascular function, and possibly positively affect other cardiovascular parameters, either directly or through improved endothelial function.

Subjects and methods

Study population

Ten females with adrenal failure were included in the study. Exclusion criteria were lactation and pregnancy, cancer, thromboembolic- and cardiovascular disease, hypertension and diabetes mellitus. Two participants chose to leave the study because of seborrhoeic side effects and anxiety, respectively. The examined study group (Addison disease, $n = 6$; monotropic ACTH deficiency, $n = 2$) had a median age of 38.5 years (range 28–52). All were on stable hydrocortisone (median 30 mg/day; range 20–40 mg/day) and fluorocortisone (0–0.2 mg/day) treatment. One of two postmenopausal patients was treated with vaginal oestradiol (vaginal capsules, oestradiol 25 mikrog). Naturally menstruating patients used contraceptive treatment (intra uterine device ($n = 4$), oral contraceptives ($n = 2$)). Other concomitant medication included thyroid hormones ($n = 1$), calcium and vitamin D ($n = 2$). None of the participants were previously treated with DHEA or androgens. Studies were performed during the early follicular phase (days 5–10) of regular cycle or in the corresponding phase of a tablet cycle, and blood was drawn in the fasting state 24 h after the last intake of tablets. The protocol was approved by the Aarhus County Ethical Scientific Committee (No. 2001 0130). All participants gave informed oral and written consent.

Study protocol

The patients were treated for 6 months with a daily morning dose of DHEA (50 mg) or placebo in a double-blind, randomized, placebo-controlled crossover design. A scheduled sequence of cardiovascular evaluation methods was performed three days before and three days just prior to ending each examination period. A wash-out period of at least 2 months was inserted before the next examination period. During the treatment period, participants continued normal daily living. The project was conducted and monitored according to the ICH-GCP guidelines (International Conference on Harmonization-Good Clinical Practice; The GCP-unit, Aarhus University Hospital). Study drug was produced according to GMP (Good Manufacturing Practice) in a licensed laboratory (Terapharm, Katwijk, Holland). Raw materials fulfil the requirements of the European Pharmacopoeia (Ph. Eur. 3rd ed.). Final analyses document the tests corresponding to the requirements for capsules in the European Pharmacopoeia. That means uniformity of content, uniformity of mass and disintegration of capsules. According to the analysis certificate the results are assay 101.5% (95–105%), mean weight variation $410.4 \text{ mg} \pm 3.6 \text{ mg}$ (401–419 mg) and disintegration 3 min (< 15 min). Figures in parenthesis indicate the requirements of the law.

Noninvasive endothelial cell function

Assessment of endothelial function in the brachial artery was performed as previously described by Celermajer *et al.*¹³ In short, changes in brachial artery diameter to reactive hyperaemia (flow-mediated dilatation (FMD)), a measure of endothelial dependent vasodilatation and to glyceryl nitrate (GTN), a measure of endothelium independent vasodilatation were measured from two-dimensional ultrasound images (Acuson 128XP/10 system with a 7.0 MHz linear array transducer, Mountain View, CA, USA) acquired 2–15 cm above the elbow. Flow velocity was measured at rest and within the first 15 s after induction of hyperaemia by means of a pulsed Doppler signal at a 70 degree angle to the vessel, with the range gate (1.5 mm) in the centre of the artery and flow calculated from Doppler flow velocity and vessel diameter. Eating and cigarette smoking were avoided 2 h before examination. Analysis of arterial recordings was done by one observer blinded to the treatment phase and the sequence of the scan.

Magnetic resonance cardiac output

Cardiac output was measured by magnetic resonance imaging (MRI) phase contrast blood velocity measurements in the ascending aorta using a Philips Intera 1.5 T scanner (Philips Medical Systems, Best, the Netherlands). Patients refrained from smoking or coffee drinking 2 h prior to scanning and rested in supine position during at least 15 min. before the cardiac output measurement. Initially stacks of scout images were obtained in three orthogonal planes using a standard steady state-free precession (SSFP) sequence. A double angulated retrospectively triggered phase contrast measurement was then performed perpendicular to the ascending aorta at the level of the main pulmonary artery. Care was taken to place the slice so as not to include the aortic valve or the aortic arch during any time point throughout the cardiac cycle. Parameters for the cardiac output scan were field of view 230 mm, matrix 256×256 (0.9 mm in-plane resolution), slice thickness 7 mm, TR/TE 12/7, velocity encoding 150 cm/s, three signal averages (NEX) giving a total scan time of approximately 6 min. Analysis was performed by automated segmentation and calculation of cardiac output using an optimized version of the algorithm as described.¹⁴

Echocardiography

All subjects underwent an echocardiographic examination performed by the same observer, with all post hoc offline analyses being done blinded to status. Echocardiograms were performed on a GE Vivid Five ultrasound machine (GE Medical System, Horten, Norway) with a 2.5 MHz transducer. Correctly orientated linear dimension measurements were made using two-dimensional imaging.¹⁵ Left ventricular mass was estimated according to the American Society of Echocardiography recommendations and based on the average of five measurements of left ventricular diameters and wall thickness in the parasternal long axis view. Concentric hypertrophy was defined as LV hypertrophy with increased ratio between wall thickness and LV cavity dimension ($2 \times$ posterior wall diameter/LV diastolic diameter > 0.43). Concentric LV remodelling was defined

as increased ratio with LV mass within normal limits. Eccentric hypertrophy was defined as LV hypertrophy without increased ratio.¹⁶ Left ventricular hypertrophy was defined as $> 47 \text{ g/m}^{2.7}$ in women.¹⁷ Fractional shortening was assessed in the parasternal long axis view; however, left ventricular volumes and ejection fraction were estimated using Simpson's modified biplane method based on three measurements.¹⁵ Endocardial border detection was enhanced by use of second harmonic imaging. Pulsed Doppler measurements were obtained with the transducer in the apical four-chamber view, with the Doppler beam aligned perpendicularly to the plane of the mitral annulus. The sample volume was placed between the tips of the mitral leaflets. Five consecutive beats during quiet respiration were used for calculating the Doppler variables. The E- and A-wave velocities as well as the isovolumetric relaxation time (IVRT) were derived from these projections. To supplement the assessment of diastolic function, but also in the advent of detecting 'pseudonormalization' of mitral inflow, assessment of the relatively load independent colour M-mode flow propagation (Vp) was performed.¹⁸ Colour M-mode flow propagation was measured in the apical four-chamber view with the M-mode cursor aligned parallel with the LV inflow. Adjustments were made to obtain the longest column of flow from the mitral annulus to the apex to avoid boundary regions. The M-mode cursor was positioned through the centre of the inflow. The velocity flow propagation was measured as the slope of the first aliasing velocity (41 cm/s) from the mitral annulus in early diastole to 4 cm distally into the ventricular cavity.¹⁹

24-h blood pressure measurement and urine collection

The 24-h AMBP was measured by Spacelab 90207 (Redmond, Washington, USA) using an oscillometric technique. Readings were obtained every 20 min. Day and night blood pressures (BPs) were calculated on hourly average values based on sleeping times applied from diaries, and 24-h urine was collected.

Assays

We measured DHEA and its sulphate (DHEAS), α -4-androstendione (A), testosterone (T), dihydrotestosterone (DHT) and 17β -oestradiol by an in-house radioimmunoassay after extraction and subsequent celite chromatography. We estimated free testosterone (fT) by a method described by Bartsch, based on measurement of sex hormone-binding globulin (SHBG), total T, and DHT, using the law of mass action, the binding constant of T and DHT to SHBG, and including a calculation of T binding to albumin (assuming a constant association constant to albumin). In this system binding to cortisol-binding globulin is thought to be negligible.²⁰ The employed method of estimating fT is essentially similar to the method suggested by Vermeulen *et al.* to be the most reliable and correlates closely with direct measurement of fT by equilibrium dialysis.²¹ We analysed SHBG by double monoclonal immunofluorometric assay (AutoDelfia, Wallac OY, Finland); intra- and interassay CV was 7.5 and 5.2%, respectively. Our inter- and intraassay coefficients of variation were as follows: SHBG 7.5%, 5.2%; T 13.8%, 8.2%; fT 6.4%, 4.7%; DHT 11.0%, 9.1%; A 11.4%, 9.4%; DHEAS 11.5%, 8.5%; and 17β -

oestradiol 10.5%, 7.4%. Cholesterol and other measures of lipid metabolism were determined on a COBAS INTEGRA (Roche, Avedøre Holme, Denmark), and creatinine by standard methods. Urine noradrenaline and adrenaline were measured by high performance liquid chromatography (HPLC) (Biorad, Herlev, Denmark).

Statistics

All statistical calculations were performed with SPSS for Windows version 10.0 (SPSS Inc., Chicago, IL, USA). Data were examined by student's two-tailed unpaired *t*-tests or the Mann-Whitney two-tailed test when appropriate. The Δ -changes from baseline to treatment (placebo or DHEA) were tested. Significance levels less than 5% were considered significant.

Results

Height, weight, BMI and waist were similar before and during placebo and DHEA treatment (height 167 ± 5 cm; weight 71 ± 12 kg; BMI $26 \pm 5 \text{ kg/m}^2$; waist 83 ± 9 cm).

Sex hormones

All patients had baseline levels of DHEA and DHEAS below or at the lowest level of normal range and responded to active treatment with normalization of DHEA and DHEAS levels. Mean levels of androstenedione, DHT, testosterone and free testosterone were all normalized. No significant changes were seen in SHBG, E2 or E1 (Table 1).

Endothelial cell function

Endothelium dependent flow-mediated dilatation (FMD) was similar during placebo and DHEA treatment and the increase in FMD was comparable to healthy persons, indicating preserved endothelial cell function in female adrenal failure. Endothelium independent dilatation after GTN was also comparable, although vessel size increased after GTN during active treatment, but decreased during placebo. Blood flow, heart rate and hyperaemia were similar in during placebo and DHEA treatment (Table 2).

Cardiac output

DHEA and placebo treatment did not influence cardiac output differently (DHEA vs. placebo, 5.1 ± 0.3 vs. 4.7 ± 0.4 l/minutes, $P = 0.3$).

Echocardiography

Neither left ventricular dimensions nor the parameters of systolic and diastolic function were affected by DHEA or placebo. Left ventricular mass mean levels were all within the normal range. However, the relative wall thickness was in all cases above 0.43, indicating that the participants had concentric left ventricular remodelling. The relative wall thickness was unaffected throughout the study (Table 3).

Table 1. Serum values (median, range) of sex hormones and sex hormone precursors in female adrenal failure before and after placebo and dehydroepiandrosterone (DHEA) treatment

	Normal range (fertile)	Placebo		DHEA		P-value*
		Start	End	Start	End	
DHEA (nmol/l)	3.8–15.9	1.74 (1.57–2.59)	2.25 (1.00–3.92)	2.11 (0.47–3.97)	10.28 (5.13–17.17)	0.01
DHEAS (nmol/l)	1200–9500	99 (99–240)	99 (99–210)	99 (99–230)	8450 (1000–19000)	0.01
Androstendione (nmol/l)	2.4–8.9	1.94 (0.27–3.91)	2.09 (0.21–3.75)	1.98 (0.18–5.15)	5.11 (1.89–9.77)	0.02
DHT (nmol/l)	0.25–1.2	0.31 (0.09–0.45)	0.27 (0.09–0.35)	0.28 (0.09–0.45)	0.83 (0.38–2.02)	0.01
Testosterone (nmol/l)	0.55–1.8	0.55 (0.20–0.71)	0.52 (0.18–0.96)	0.49 (0.16–1.50)	1.30 (0.69–2.65)	0.01
Free testosterone (nmol/l)	0.006–0.034	0.007 (0.002–0.012)	0.007 (0.003–0.013)	0.004 (0.002–0.019)	0.017 (0.010–0.032)	0.02
17 β -oestradiol (pmol/l)	< 40–400	155 (39–540)	195 (39–1100)	290 (39–630)	220 (39–630)	0.3
Oestrone (pmol/l)	70–270	97 (39–270)	140 (39–340)	175 (39–440)	155 (77–410)	0.7
SHBG (nmol/l)	41–170	88 (27–114)	96 (37–156)	78 (36–163)	79 (26–144)	0.2

*P-value: the change from start to end during the two treatment arms (placebo or dehydroepiandrosterone (DHEA)) was tested.

Table 2. Noninvasive endothelial cell function in female adrenal failure before and after placebo and dehydroepiandrosterone (DHEA) treatment

	Placebo		DHEA		P-value*
	Start	End	Start	End	
Vessel size (mm) baseline resting	2.50 \pm 0.11	2.53 \pm 0.09	2.49 \pm 0.11	2.60 \pm 0.08	0.5
Vessel size (mm) after cuff deflation	2.80 \pm 0.10	2.73 \pm 0.08	2.71 \pm 0.08	2.82 \pm 0.08	0.1
Vessel size (mm) resting intermediary	2.57 \pm 0.11	2.53 \pm 0.09	2.44 \pm 0.07	2.56 \pm 0.07	0.1
Vessel size (mm) after GTN	3.15 \pm 0.09	3.03 \pm 0.06	3.03 \pm 0.06	3.19 \pm 0.09	0.009
Flow mediated dilatation (%)	112.1 \pm 2.2	108.0 \pm 1.6	108.9 \pm 1.5	108.6 \pm 1.1	0.3
GTN induced dilatation (%)	126.7 \pm 3.4	123.0 \pm 2.7	125.5 \pm 2.4	124.6 \pm 1.6	0.4
Flow (ml/min) baseline	0.065 \pm 0.018	0.072 \pm 0.020	0.072 \pm 0.020	0.052 \pm 0.012	0.4
Heart rate baseline	75 \pm 4	76 \pm 4	73 \pm 4	79 \pm 4	0.2
Flow (ml/min) after cuff deflation	0.214 \pm 0.017	0.258 \pm 0.033	0.209 \pm 0.023	0.195 \pm 0.019	0.7
Heart rate after cuff deflation	76 \pm 4	75 \pm 4	79 \pm 4	82 \pm 3	0.2
Flow (ml/min) resting intermediary	0.056 \pm 0.017	0.054 \pm 0.018	0.036 \pm 0.008	0.034 \pm 0.007	0.8
Heart rate resting intermediary	73 \pm 3	75 \pm 4	74 \pm 4	75 \pm 3	0.4
Flow (ml/min) after GTN	0.040 \pm 0.008	0.050 \pm 0.016	0.043 \pm 0.011	0.031 \pm 0.004	0.4
Heart rate after GTN	86 \pm 3	86 \pm 3	83 \pm 4	82 \pm 4	1.0
Hyperaemia (% increase in flow) after cuff deflation	486 \pm 124	549 \pm 146	533 \pm 126	542 \pm 113	0.7

*P-value: the change from start to end during the two treatment arms (placebo or DHEA) was tested.
GTN, glyceryl nitrate.

24 blood pressure monitoring

DHEA treatment did not influence 24-h blood pressure, heart rate or pulse amplitude and no alterations in day-night distribution were detected (Table 4, Fig. 1).

Urine production and catecholamines

Twenty-four-hour urine production (DHEA vs. placebo 2330 \pm 184 vs. 2103 \pm 188 ml, $P = 0.1$) and creatinine clearance (58 \pm 6 vs. 52 \pm 3 ml/min, $P = 0.5$) was not affected by treatment. Urine adrenaline excretion was undetectable in two patients at baseline, three patients after placebo treatment and in seven patients after DHEA treatment, but levels were not statistically different (results not

shown). Urine noradrenaline excretion was not affected by treatment (results not shown).

Lipids

No significant changes in total cholesterol, HDL, LDL or triglycerides were seen (Table 5).

Discussion

Results from the present clinical randomized controlled study are essentially negative, and thus supplementation with DHEA for 6 months in physiological relevant doses does not seem to impact cardiovascular or endothelial variables in hypoadrenal women

Table 3. Echocardiographic features and systolic and diastolic filling parameters in women with adrenal failure before and after placebo or dehydroepiandrosterone (DHEA) treatment

	Placebo		DHEA		P-value*
	Start	End	Start	End	
Structural characteristics					
LV end-diastolic diameter (cm)	4.49 ± 0.18	4.35 ± 0.14	4.45 ± 0.20	4.58 ± 0.19	0.2
LV end-systolic diameter (cm)	2.18 ± 0.46	2.19 ± 0.46	2.01 ± 0.42	2.17 ± 0.47	0.4
LV intraventricular septum (cm)	0.80 ± 0.03	0.71 ± 0.05	0.76 ± 0.06	0.72 ± 0.06	0.5
LV posterior wall thickness (cm)	1.72 ± 0.43	1.66 ± 0.43	1.85 ± 0.49	1.80 ± 0.50	0.9
LV mass index (g/m ²)	102 ± 8	85 ± 6	91 ± 8	96 ± 10	0.1
End-diastolic relative wall thickness	0.77 ± 0.19	0.75 ± 0.19	0.80 ± 0.19	0.77 ± 0.20	0.8
Systolic performance					
Fractional shortening (%)	30.75 ± 1.81	30.00 ± 1.95	31.63 ± 1.75	29.46 ± 1.03	0.4
Ejection fraction	66.13 ± 2.35	62.88 ± 2.52	62.63 ± 2.47	63.00 ± 1.83	0.2
Diastolic performance					
Mitral E-wave velocity (cm/s)	75.50 ± 7.09	74.75 ± 4.03	76.75 ± 3.30	78.88 ± 5.20	0.8
Mitral A-wave velocity (cm/s)	54.88 ± 4.68	52.25 ± 4.54	56.25 ± 4.60	61.63 ± 5.11	0.3
E/A ratio	1.26 ± 0.10	1.47 ± 0.13	1.41 ± 0.11	1.32 ± 0.09	0.2
Deceleration time (ms)	202.63 ± 15.98	185.88 ± 15.28	172.25 ± 9.36	186.25 ± 10.04	0.2
Isovolumetric relaxation time (ms)	80.00 ± 3.16	75.29 ± 3.48	77.57 ± 3.02	72.43 ± 4.60	0.9
Velocity flow propagation (cm/s)	65.00 ± 3.21	55.88 ± 3.81	57.50 ± 3.55	59.75 ± 5.03	0.02

Data are means ± SEM. *P-value: the change from start to end during the two treatment arms (placebo or DHEA) was tested. LV, left ventricle.

Table 4. 24-h ambulatory blood pressure measurements in women with adrenal failure before and after placebo or dehydroepiandrosterone (DHEA) treatment

		Placebo		Dehydroepiandrosterone (DHEA)		P-value*
		Start	End	Start	End	
24-h	Systolic	108.0 ± 3.3	110.9 ± 5.1	109.7 ± 4.0	112.5 ± 3.9	1.0
	Diastolic	73.8 ± 3.0	75.8 ± 3.9	74.7 ± 3.2	73.8 ± 2.9	0.3
	Pulse	76.3 ± 3.3	75.2 ± 2.2	76.9 ± 3.1	72.9 ± 2.7	0.3
Day	Systolic	112.4 ± 3.5	116.3 ± 5.3	111.2 ± 4.6	115.1 ± 4.6	1.0
	Diastolic	78.3 ± 2.9	80.7 ± 4.1	78.2 ± 2.9	79.1 ± 3.4	0.5
	Pulse	84.6 ± 4.6	81.2 ± 3.6	83.2 ± 3.8	80.0 ± 3.2	1.0
Night	Systolic	98.3 ± 3.7	101.1 ± 5.0	102.2 ± 5.1	107.7 ± 8.6	0.7
	Diastolic	65.0 ± 2.9	66.9 ± 3.5	64.1 ± 2.9	63.6 ± 2.4	0.3
	Pulse	66.1 ± 3.8	66.9 ± 1.9	65.2 ± 3.1	63.8 ± 2.8	0.6
Day/night systolic ratio	1.15 ± 0.02	1.14 ± 0.02	1.09 ± 0.06	1.09 ± 0.07	0.9	
Day/night diastolic ratio	1.21 ± 0.04	1.20 ± 0.03	1.24 ± 0.03	1.25 ± 0.03	0.4	

Data are means ± SEM. *P-value: the change from start to end during the two treatment arms (placebo or DHEA) was tested.

	Placebo		DHEA		P-value*
	Start	End	Start	End	
Triglyceride (mmol/l)	1.63 ± 0.38	1.45 ± 0.30	1.38 ± 0.27	1.24 ± 0.23	0.5
Cholesterol (mmol/l)	5.68 ± 0.54	5.33 ± 0.45	5.28 ± 0.34	5.23 ± 0.37	0.8
HDL (mmol/l)	1.50 ± 0.09	1.50 ± 0.09	1.68 ± 0.13	1.74 ± 0.11	0.6
LDL (mmol/l)	3.44 ± 0.42	3.16 ± 0.37	2.99 ± 0.28	2.95 ± 0.31	0.9

Data are mean ± SEM. *P-value: the change from start to end during the two treatment arms (placebo or DHEA) was tested.

Table 5. Lipids before and after placebo and dehydroepiandrosterone (DHEA) treatment in female adrenal failure

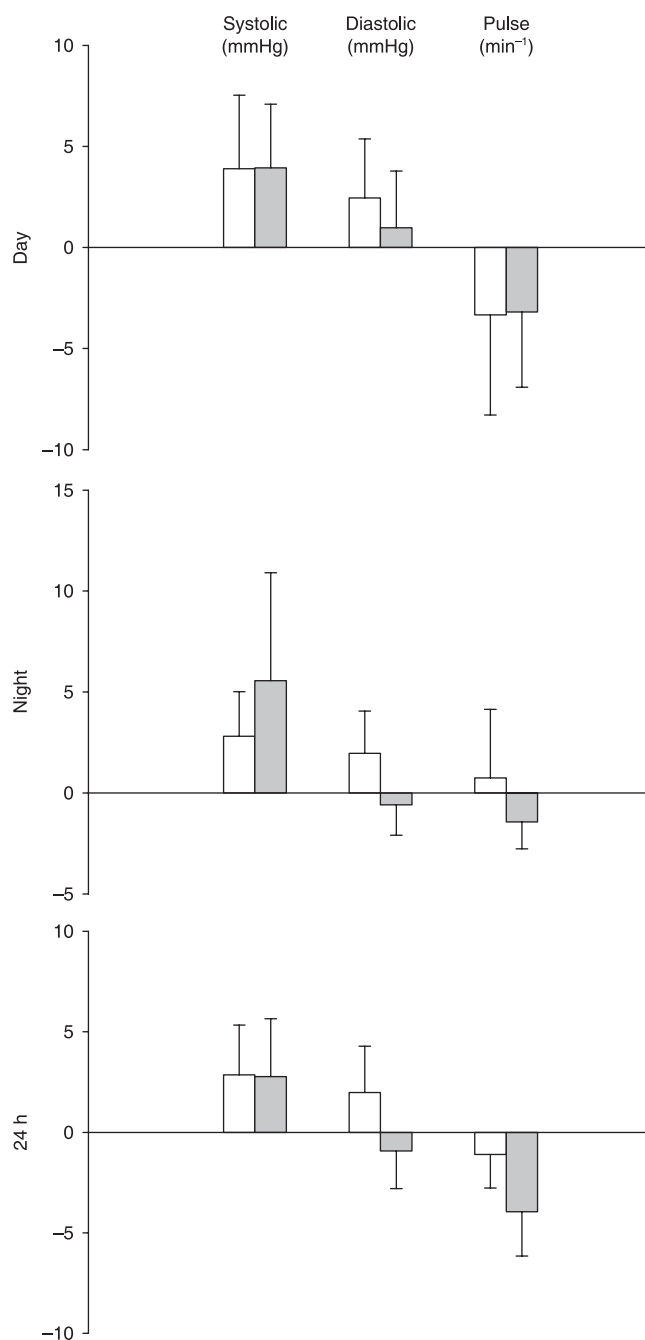


Fig. 1 Delta values of day time, night time and total 24-h blood pressure and heart rate after 6 months treatment with placebo (white) and dehydroepiandrosterone (DHEA, grey) in female adrenal failure.

receiving stable substitution therapy. We thoroughly evaluated cardiovascular effects in eight women with adrenal insufficiency and low levels of adrenal androgen precursors. The parallel substitution treatment with DHEA in physiological doses for six months normalized circulating androgen levels, but did not influence performance, dynamics or structural appearance of the cardiovascular system.

DHEA substitution therapy in adrenal insufficiency is still a controversial issue, but due to the fact that a cellular receptor for DHEA

seems to be present in endothelial tissues, with the ability to actively synthesize NO¹¹ an effect that is nongenomic and mediated via phosphatidylinositol (PI) 3-kinase-dependent pathways and that it also affects the secretion of the vasoconstrictor endothelin-1 via mitogen-activated protein kinase (MAPK)-dependent pathways²² it is of considerable interest to clarify any possible effect on the cardiovascular system. In a study of 36 healthy postmenopausal women treated with 100 mg of DHEA for 3 months in a parallel design, DHEA increased flow-mediated dilatation, improved microvascular reactivity and reduced total cholesterol, and increased *in vitro* expression of endothelial NO and activity of extracellular signal-regulated kinase (ERK) 1/2.¹² All these changes pointed towards a significant effect of DHEA on endothelial function, and since the *in vitro* effects could not be blocked by selective testosterone and oestradiol receptor blockers, lead the investigators to suggest an independent DHEA receptor were mediating the effects. In a study of hyperlipidaemic middle-aged males DHEA improved flow-mediated dilatation and lowered fasting plasma glucose.²³ Our results are clearly discordant and although we studied a somewhat younger group of women suffering from adrenal failure we did not see even a trend towards any discernible effects of DHEA treatment on endothelial function. Circulating androgens normalized during treatment with 50-mg DHEA with testosterone increasing by 250%, while in the study by Williams *et al.* the increase in serum testosterone was in the order of 400% after 100 mg of DHEA, and it may be that the effect seen in the latter study was due to this large increase in testosterone.¹² In the study of Kawano *et al.* the male participants receiving active treatment experienced a four-fold increase in circulating DHEA, but no change in testosterone.²³ Our study group was smaller, but the fact that we did not see even a trend towards an effect, makes it less likely that we are witnessing a type 2 error. Of note, the two studies mentioned above used a parallel design, while we used a crossover design, perhaps explaining the discordant results. In a study of a mixed group of hypoadrenal women (Addison's disease, adrenalectomized patients with Cushing's disease, and others), Dhataria *et al.* demonstrated improved insulin sensitivity and decreases in total cholesterol, as well as HDL, LDL and triglycerides during 12 weeks of DHEA treatment,²⁴ whereas we did not see any change in cholesterol during active treatment. Williams *et al.* also recorded a decrease in total cholesterol in healthy postmenopausal women,¹² while Kawano *et al.* did not find any changes in cholesterol in middle-aged males, which is in accordance with our data.²³ In a longitudinal study of older women, DHEAS did not predict overall mortality.²⁵

The extensive assessment of both left ventricular systolic and diastolic function and cardiac output by MRI implicates that DHEA in physiological doses does not influence left ventricular structure or performance. However, it was quite surprising to find that all included patients had concentric remodelling of the left ventricle, despite the young age of participants and the absence of hypertension or other concomitant disease. Fallo *et al.* described reduced left ventricular dimensions in untreated patients with Addison's disease, which normalized after substitution²⁶ and Allolio *et al.* found impaired left diastolic relaxation after 48 h of withdrawal of glucocorticoid therapy.²⁷ Aldosterone has trophic effects on the myocardium, but its role in the nonfailing heart seems modest.²⁸ On the contrary it seems likely that supplementation of hydrocortisone

which is inherently difficult and often leads to supraphysiological doses may have an impact on the left ventricular geometry. Our findings of an altered LV geometry with normal LV performance are similar to the effects of high doses of hydrocortisone on the myocardium in an experimental setting (i.e. sheep)²⁹ and to what has been found in Cushing's syndrome³⁰ although several variables, such as poorly controlled hypertension, diabetes and overweight may have confounded the results from the latter study.

We did not record any change in 24-h ambulatory blood pressure measurements during active treatment. Longitudinal studies have suggested a link between low DHEA and subsequent development of cardiovascular disease.³¹ In a similar study design in a group of eight patients with Turner syndrome and resultant severe hypogonadism, we detected profound changes in 24-h ambulatory blood pressure during active treatment with 17 β -oestradiol³² again suggesting that the lack of change in blood pressure in the present study can be interpreted as lack of clinical significant effect of DHEA supplementation on blood pressure, and not dismissed as a type 2 error.

The design of the present pilot study, however, has limitations. The study group was small and we had two dropouts. This may lead to a type 2 error when analysing data, and we cannot exclude such an error. On the other hand, we did not find even a trend in the data towards a positive effect of DHEA treatment on a range of cardiovascular parameters. In addition, the expected effect size of the measured variables may also preclude definite conclusions. For instance, the changes in endothelial function during placebo and DHEA treatment were variable. Thus, exclusion of a physiological effect of DHEA may not be possible from the present data, but we may be able to conclude that we could not find any physiologically relevant effect of DHEA treatment in hypoadrenal women.

We conclude that restoration of circulating levels of androgens to physiological levels with DHEA does not affect endothelial and cardiovascular parameters in hypoadrenal women.

Acknowledgements

Jens Juel Christiansen is supported by a PhD research fellowship by the University of Aarhus, Denmark. We thank Elsebeth Hornemann, Hanne Pedersen, Lone Svendsen and Bente Mortensen for expert technical help. The study was supported by a grant from the Danish Health Research Council, grant no. 9600822 (Århus University – Novo Nordisk Center for Research in Growth and Regeneration).

References

- Labrie, F., Luu-The, V., Labrie, C., Belanger, A., Simard, J., Lin, S.X. & Pelletier, G. (2003) Endocrine and intracrine sources of androgens in women: inhibition of breast cancer and other roles of androgens and their precursor dehydroepiandrosterone. *Endocrine Reviews*, **24**, 152–182.
- Young, J., Couzinet, B., Nahoul, K., Brailly, S., Chanson, P., Baulieu, E.E. & Schaison, G. (1997) Panhypopituitarism as a model to study the metabolism of dehydroepiandrosterone (DHEA) in humans. *Journal of Clinical Endocrinology and Metabolism*, **82**, 2578–2585.
- Arlt, W., Callies, F., van Vlijmen, J.C., Koehler, I., Reincke, M., Bidlingmaier, M., Huebler, D., Oettel, M., Ernst, M., Schulte, H.M. & Allolio, B. (1999) Dehydroepiandrosterone replacement in women with adrenal insufficiency. *New England Journal of Medicine*, **341**, 1013–1020.
- Hunt, P.J., Gurnell, E.M., Huppert, F.A., Richards, C., Prevost, A.T., Wass, J.A., Herbert, J. & Chatterjee, V.K. (2000) Improvement in mood and fatigue after dehydroepiandrosterone replacement in Addison's disease in a randomized, double-blind trial. *Journal of Clinical Endocrinology and Metabolism*, **85**, 4650–4656.
- Gebre-Medhin, G., Husebye, E.S., Mallmin, H., Helstrom, L., Berne, C., Karlsson, F.A. & Kampe, O. (2000) Oral dehydroepiandrosterone (DHEA) replacement therapy in women with Addison's disease. *Clinical Endocrinology*, **52**, 775–780.
- Lovas, K., Gebre-Medhin, G., Trovik, T.S., Fougner, K.J., Uhlving, S., Nedrebo, B.G., Myking, O.L., Kampe, O. & Husebye, E.S. (2003) Replacement of dehydroepiandrosterone in adrenal failure: no benefit for subjective health status and sexuality in a 9-month, randomized, parallel group clinical trial. *Journal of Clinical Endocrinology and Metabolism*, **88**, 1112–1118.
- Johannsson, G., Burman, P., Wiren, L., Engstrom, B.E., Nilsson, A.G., Ottosson, M., Jonsson, B., Bengtsson, B.A. & Karlsson, F.A. (2002) Low dose dehydroepiandrosterone affects behavior in hypopituitary androgen-deficient women: a placebo-controlled trial. *Journal of Clinical Endocrinology and Metabolism*, **87**, 2046–2052.
- Roth, G.S., Lane, M.A., Ingram, D.K., Mattison, J.A., Elahi, D., Tobin, J.D., Muller, D. & Metter, E.J. (2002) Biomarkers of caloric restriction may predict longevity in humans. *Science*, **297**, 811.
- Moriyama, Y., Yasue, H., Yoshimura, M., Mizuno, Y., Nishiyama, K., Tsunoda, R., Kawano, H., Kugiyama, K., Ogawa, H., Saito, Y. & Nakao, K. (2000) The plasma levels of dehydroepiandrosterone sulfate are decreased in patients with chronic heart failure in proportion to the severity. *Journal of Clinical Endocrinology and Metabolism*, **85**, 1834–1840.
- Nestler, J.E., Clore, J.N. & Blackard, W.G. (1992) Dehydroepiandrosterone: the missing link between hyperinsulinemia and atherosclerosis? *FASEB Journal*, **6**, 3073–3075.
- Liu, D. & Dillon, J.S. (2002) Dehydroepiandrosterone activates endothelial cell nitric-oxide synthase by a specific plasma membrane receptor coupled to Galpha (i2,3). *Journal of Biological Chemistry*, **277**, 21379–21388.
- Williams, M.R., Dawood, T., Ling, S., Dai, A., Lew, R., Myles, K., Funder, J.W., Sudhir, K. & Komesaroff, P.A. (2004) Dehydroepiandrosterone increases endothelial cell proliferation *in vitro* and improves endothelial function *in vivo* by mechanisms independent of androgen and estrogen receptors. *Journal of Clinical Endocrinology and Metabolism*, **89**, 4708–4715.
- Celermajer, D.S., Sorensen, K.E., Gooch, V.M., Spiegelhalter, D.J., Miller, O.I., Sullivan, I.D., Lloyd, J.K. & Deanfield, J.E. (1992) Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet*, **340**, 1111–1115.
- Kozerke, S., Botnar, R., Oyre, S., Scheidegger, M.B., Pedersen, E.M. & Boesiger, P. (1999) Automatic vessel segmentation using active contours in cine phase contrast flow measurements. *Journal of Magnetic Resonance Imaging*, **10**, 41–51.
- Schiller, N.B., Shah, P.M., Crawford, M., DeMaria, A., Devereux, R., Feigenbaum, H., Gutgesell, H., Reichek, N., Sahn, D. & Schnittger, I. (1989) Recommendations for quantitation of the left ventricle by two-dimensional echocardiography: American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. *Journal of the American Society of Echocardiography*, **2**, 358–367.
- Roman, M.J., Pickering, T.G., Schwartz, J.E., Pini, R. & Devereux, R.B. (1996) Relation of arterial structure and function to left ventricular

- geometric patterns in hypertensive adults. *Journal of the American College of Cardiology*, **28**, 751–756.
- 17 de Simone, G., Devereux, R.B., Daniels, S.R., Koren, M.J., Meyer, R.A. & Laragh, J.H. (1995) Effect of growth on variability of left ventricular mass: assessment of allometric signals in adults and children and their capacity to predict cardiovascular risk. *Journal of the American College of Cardiology*, **25**, 1056–1062.
 - 18 Moller, J.E., Poulsen, S.H., Sondergaard, E. & Egstrup, K. (2000) Preload dependence of color M-mode Doppler flow propagation velocity in controls and in patients with left ventricular dysfunction. *Journal of the American Society of Echocardiography*, **13**, 902–909.
 - 19 Takatsuji, H., Mikami, T., Urasawa, K., Teranishi, J., Onozuka, H., Takagi, C., Makita, Y., Matsuo, H., Kusuoka, H. & Kitabatake, A. (1996) A new approach for evaluation of left ventricular diastolic function: spatial and temporal analysis of left ventricular filling flow propagation by color M-mode Doppler echocardiography. *Journal of the American College of Cardiology*, **27**, 365–371.
 - 20 Bartsch, W. (1980) Interrelationships between sex hormone-binding globulin and testosterone, 5 alpha-dihydrotestosterone and oestradiol-17 beta in blood of normal men. *Maturitas*, **2**, 109–118.
 - 21 Vermeulen, A., Verdonck, L. & Kaufman, J.M. (1999) A critical evaluation of simple methods for the estimation of free testosterone in serum. *Journal of Clinical Endocrinology and Metabolism*, **84**, 3666–3672.
 - 22 Formoso, G., Chen, H., Kim, J.A., Montagnani, M., Consoli, A. & Quon, M.J. (2006) Dehydroepiandrosterone mimics acute actions of insulin to stimulate production of both nitric oxide and endothelin 1 via distinct phosphatidylinositol 3-kinase- and mitogen-activated protein kinase-dependent pathways in vascular endothelium. *Molecular Endocrinology*, **20**, 1153–1163.
 - 23 Kawano, H., Yasue, H., Kitagawa, A., Hirai, N., Yoshida, T., Soejima, H., Miyamoto, S., Nakano, M. & Ogawa, H. (2003) Dehydroepiandrosterone supplementation improves endothelial function and insulin sensitivity in men. *Journal of Clinical Endocrinology and Metabolism*, **88**, 3190–3195.
 - 24 Dhataria, K., Bigelow, M.L. & Nair, K.S. (2005) Effect of dehydroepiandrosterone replacement on insulin sensitivity and lipids in hypoadrenal women. *Diabetes*, **54**, 765–769.
 - 25 Barrett-Connor, E. & Goodman-Gruen, D. (1995) Dehydroepiandrosterone sulfate does not predict cardiovascular death in postmenopausal women: The Rancho Bernardo Study. *Circulation*, **91**, 1757–1760.
 - 26 Fallo, F., Betterle, C., Budano, S., Lupia, M., Boscaro, M. & Sonino, N. (1999) Regression of cardiac abnormalities after replacement therapy in Addison's disease. *European Journal of Endocrinology*, **140**, 425–428.
 - 27 Allolio, B., Ehses, W., Steffen, H.M. & Muller, R. (1994) Reduced lymphocyte beta 2-adrenoceptor density and impaired diastolic left ventricular function in patients with glucocorticoid deficiency. *Clinical Endocrinology*, **40**, 769–775.
 - 28 Connell, J.M. & Davies, E. (2005) The new biology of aldosterone. *Journal of Endocrinology*, **186**, 1–20.
 - 29 Lumbers, E.R., Boyce, A.C., Joulianos, G., Kumarasamy, V., Barner, E., Segar, J.L. & Burrell, J.H. (2005) Effects of cortisol on cardiac myocytes and on expression of cardiac genes in fetal sheep. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology*, **288**, R567–R574.
 - 30 Muiesan, M.L., Lupia, M., Salvetti, M., Grigoletto, C., Sonino, N., Boscaro, M., Rosei, E.A., Mantero, F. & Fallo, F. (2003) Left ventricular structural and functional characteristics in Cushing's syndrome. *Journal of the American College of Cardiology*, **41**, 2275–2279.
 - 31 Hougaku, H., Fleg, J.L., Najjar, S.S., Lakatta, E.G., Harman, S.M., Blackman, M.R. & Metter, E.J. (2006) Relationship between androgenic hormones and arterial stiffness, based on longitudinal hormone measurements. *American Journal of Physiology. Endocrinology and Metabolism*, **290**, E234–E242.
 - 32 Gravholt, C.H., Hansen, K.W., Erlandsen, M., Ebbelohj, E. & Christiansen, J.S. (2006) Nocturnal hypertension and impaired sympathovagal tone in Turner syndrome. *Journal of Hypertension*, **24**, 353–360.