Long-term dehydroepiandrosterone substitution in female adrenocortical failure, body composition, muscle function and bone metabolism – a randomized trial

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Short title: DHEA and body composition

No conflicts of interest.

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Abstract

Context: Adrenal derived androgens are low in women with adrenal failure. The physiological consequences of substitution therapy are uncertain.

Objective: To investigate the effects of dehydroepiandrosterone (DHEA) substitution in women with adrenal failure on body composition, fuel metabolism, and inflammatory markers

Design, Participants and Intervention: 10 female patients (median age 38.5 years, range 28-52) with adrenal failure were treated with DHEA 50 mg for 6 months in a double blind, randomised, placebo controlled, crossover study. The participants underwent DXA scan, CT scan of abdominal fat, indirect calorimetry, bicycle ergometry, muscle and fat biopsies, and blood samples.

Results: Baseline androgens were normalized to fertile range during active treatment. Anthropometric data were unaffected, but lean body mass (LBM) slightly increased compared to placebo (delta LBM (kg) placebo vs. DHEA: -0.48±6.1 vs.1.6±3.4, p=0.02) with no alterations in total or abdominal fat mass. PTH increased with DHEA, but no significant changes were observed in other bone markers or in bone mineral content. The mRNA levels of markers of tissue inflammation (adiponectin, IL-6, IL-10, MCP-1, and TNF-α) in fat and muscle tissue were unaffected by DHEA treatment, as was indirect calorimetry and maximal oxygen uptake. A high proportion of self-reported seborrhoic side effects were recorded (60%).

Conclusion: In female adrenal failure normalization of androgens with DHEA 50 mg for 6 months had no effects in muscle, fat and bone tissue and in fuel metabolism in this small study. A small increase in LBM was seen. Treatment was associated with a high frequency of side effects.
Introduction

The androgen receptor, being a member of the nuclear receptor superfamily, is responsible for mediating development of primary and secondary sex characteristics during foetal life, childhood and puberty and for maintaining these features during adult life. The beneficial impact of endogenous testosterone as well as testosterone replacement in hypogonadal males on maintenance of bone (1;2), muscle mass (2;3), body fat distribution, and quality of life (4) is well documented. Cessation of androgen secretion in the adult female is seen in adrenal insufficiency where adrenal androgen precursor production is blunted or almost absent (5;6). Substitution therapy with an oral dose of 50 mg of the adrenal androgen dehydroepiandrosterone (DHEA) is followed by normalization of the spectrum of circulating androgens including testosterone (7-9). Questionnaires in controlled clinical trials have shown some (9-12) or no (13-15) effects on quality of life, and a high proportion of self reported skin related side effects (9-11;13;14). Substantial physiological alterations upon androgen substitution with DHEA in female adrenocortical failure are still controversial and probably obscured by the associated hormone deficiencies (ie. hypocortisolism with or without other aspects of hypopituitarism), and it remains to be determined whether DHEA has any effects on muscle and fat distribution.

We therefore evaluated the influence of DHEA substitution in female adrenal failure on muscle, fat and bone tissue as well as indicators of fuel metabolism. We took biopsies from both fat and muscle which were analyzed for expression of enzymes and proteins known to be involved in hormonal and inflammatory pathways, and determined muscle and fat distribution by DXA scan and abdominal CT scan. A daily dose of 50 mg DHEA was administrated for 6 months in a cross over randomised and placebo controlled trail.
Subjects and methods

Ten females with adrenocortical failure were included in the study. Exclusion criteria were lactation and pregnancy, neoplastic-, thromboembolic- and cardiovascular diseases and diabetes mellitus. Two participants discontinued the study because of seborrhoic side effects and anxiety. The examined study group (Addison disease, n= 6; monoprote ACTH deficiency, n= 2) had a median age of 38.5 years (range 28-52). All were on stable hydrocortisone (20 - 40mg/day) and fluorocortisone (0 - 0.2mg/day) treatment. One of two postmenopausal patients was treated with vaginal estradiol (vaginal capsules, estradiol 25 µg). Naturally menstruating patients used contraceptives (4 intra uterine devices, 2 combined oral contraceptives). Other concomitant medication included thyroid hormones (1 patient), calcium and vitamin D (2 patients). None of the participants were previously treated with DHEA or androgens. Studies were performed during the early follicular phase (day 5-10) of regular cycle or in the corresponding phase of a tablet cycle. The protocol was approved by the Aarhus County Ethical Scientific committee (no.2001 0130). All participants gave informed oral and written consent. The clinical trial was registered at ClinicalTrials.gov (NCT00471900). Cardiovascular data from this study have previously been published (16).

Study protocol

The patients were treated for 6 months with a daily morning dose of DHEA (50 mg) or placebo in a double-blind, randomised, placebo-controlled crossover design. Blinding and randomization was performed by the Hospital Pharmacy in blocks of 5. Everybody else was blinded to this procedure until the GCP unit had finalized monitoring the study and the randomization code was broken. A washout 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). The study drug was produced according to GMP (Good Manufacturing Practice) in a licensed laboratory (Terapharm, Katwijk, Holland). Raw materials fulfill the requirements of the European Pharmacopea (Ph. Eur. 3rd ed.). Final analyses document the tests corresponding to the requirements for capsules in the European Pharmacopea. That means uniformity of capsule content, uniformity of capsule mass and disintegration of capsules. According to the analysis certificate the results are assay 101.5% (95–105%), mean weight variation 410.4±3.6 mg (401–419 mg) and disintegration 3 min (< 15 min). Figures in parenthesis indicate the requirements of the law. Physical examination consisted of anthropometrics, muscle strength, ergometry, indirect calorimetry and CT scan of abdominal fat distribution. Blood samples included routine biochemistry, bone markers, sex hormones and regulatory hormones. This program was performed in similar sequence prior to and at the end of each treatment period. Muscle and fat biopsy and DEXA scan were done at the end of each study period. At baseline (t=120–150 min), percutaneous muscle biopsies from the quadriceps muscle were performed after injecting local anesthesia (10% lidocaine) in the skin and percutaneous regions. A small incision was made at the lateral aspect of the thigh in the midbelly of the vastus lateralis of the quadriceps muscle. Using a modified Bergstrom needle (5 mm), muscle tissue was obtained by suction. The biopsy was immediately put in liquid nitrogen and kept at –80°C. Fat biopsies were taken from the subcutaneous abdominal adipose tissue depot (periumbilically). Using local anesthesia (5 mg/ml lidocaine), the biopsies were taken by needle aspiration (liposuction). The adipose tissue was washed thoroughly with isotonic saline and then frozen in liquid nitrogen for later RNA extraction.
Determination of adipokine mRNA levels

In the fat tissue biopsies, mRNA expression of adiponectin, interleukin (IL)-6, IL-10, tumor necrosis factor (TNF)-α, monocyte chemoattratrant protein (MCP)-1, and 11β-HSD were assessed. In accordance with tissue specificity only mRNA expression of IL-6, TNF-α, and MCP-1 were assessed in the muscle tissue biopsies.

For mRNA determination the following oligonucleotide primer pairs were used.
adiponectin: 5'CATGACCAGGAAACCACGACT3'; 5'TGAATGCTGAGCGGTAT3', IL-6: 5'AAATGCCAGCCTGCTGACGAAG3'; 5'AACAACAATCTGAGGTGCCCATGCTAC3', IL-10: 5'ATGGAGCGAAGGT-TAGTGGTCA3'; 5'CTCGCTTTAATTGTATGTATGCT3', TNF-α: 5'CGAGTGACAAGCCTGTAGC3'; 5'GGTGTGGGTGAGGAGCACAT3', MCP-1: 5'CAATCAATGCCCATGACAT3'; 5'GATTCTTTGGGTTGAGT3', 11β-HSD: 5'GACCATGACCTTGCGAGCATTGT3'; 5'GACGCCAAGAACACAGAGTGATTGG3' and β-actin: 5'ACGGGGTCAACCCCACCTGTGC 3'; 5'CTAGAAGGATTTTCGCGTGGACGATG3'. RNA was isolated from ~250 mg of adipose tissue using the TriZol Reagent (Invitrogen, Carlsbad, CA). RNA was quantified by measuring absorbancy at 260 and 280 nm. The integrity of the RNA was checked by visual inspection of the two ribosomal RNAs, 18S and 28S, on an agarose gel. RNA was reversely transcribed with RT and random hexamer primers at 23 °C for 10 minutes, 42 °C for 60 minutes, and 95 °C for 10 minutes according to the manufacturer’s instructions (GeneAmp RNA PCR kit, PerkinElmer Life and Analytical Sciences, Boston, MA). Then 2 µl of each RT reaction was amplified in PCR-mastermix containing the specific primers, Hot Star Taq DNA polymerase, and SYBR-Green PCR buffer. All samples were determined as duplicates. Real-time quantification of target gene (adiponectin, IL-6, IL-10, TNF-α, MCP-1, 11β-HSD) relative to housekeeping gene (β-actin) was performed using a SYBR-Green real-time PCR assay and an iCycler PCR machine.
(Bio-Rad, Hercules, CA). In brief, samples were incubated in separate tubes for initial denaturation at 95 °C for 10 minutes, followed by 40 PCR amplification cycles. Each cycle consisted of 30 seconds at 95 °C, 30 seconds at 57 °C and extension for 60 seconds at 74 °C. During final extension increasing fluorescence was measured in real time. Data was obtained as threshold cycle values (Ct = the cycle number at which fluorescence reached 10 times the SD of the background fluorescence). Relative gene expression of target gene to β-actin was calculated as described in the User Bulletin No. 2, 1997 from Perkin Elmer Life and Analytical Sciences (Perkin Elmer Cetus, Norwalk CT, USA).

**DXA and CT Scans**

BMC (grams) and area-adjusted BMD (aBMD) (grams/square centimeter) were measured at the lumbar spine (L2–L4), the hip (femoral neck and trochanteric region), and the nondominant forearm (ultradistal and proximal part of distal third) by DXA on Hologic 1000/w or 2000/w osteodensitometers (Hologic, Inc., Waltham, MA). Cross-calibration was ensured through the use of double measurements and a phantom. Precision for BMD was 1.5% for the lumbar spine, 2.1% for the femoral neck, and 1.9% for the ultradistal forearm. These quantities included cross-over calibration, change of hardware, change of technicians, and long-term stability (<0.2%/yr). The amounts of intra-abdominal (visceral) and subcutaneous fat, as well as total abdominal area was determined by computerized tomography (CT) with a Somatom Plus-S scanner. The subjects were studied in the supine position. The area scanned comprised a 10-mm cross-sectional slice at the umbilicus, using 120 kV and 330 mA. The same technician performed all the scans, which subsequently were analyzed blindly by the same radiologist.

*Indirect calorimetry, muscle strength and maximal oxygen uptake*
Indirect calorimetry (Deltatrac metabolic monitor; Datex, Helsinki, Finland) was performed for 30 min in basal state. The initial 5 min were used for acclimatization, and calculations of respiratory exchange ratio (RER) and resting energy expenditure (EE) were based on mean values of 25 measurements of 1 min each. Likewise, rates of protein lipid and glucose oxidation were estimated after correction for urinary excretion of urea (17). Maximal oxygen consumption (VO$_{2\text{max}}$) test was performed on a bicycle ergometer using a standardised protocol. The initial workload was increased with 10 watts every 30 seconds until exhaustion. Breath-by-breath gas exchange analysis was performed and maximal oxygen consumption was determined as the highest O$_2$ consumption achieved during exercise with a calorimeter (Jaeger Oxycon Delta, Erich Jaeger, Hoechberg, Germany), and VO$_{2\text{max}}$ was calculated. The isometric strength of the right biceps brachialis and quadriceps muscles was assessed by means of a dynamometer (Good strength, Metitur Ltd, Jyväskylä, Finland), which electronically measures the isometric muscle functions in the upper and lower limbs. The strength was calculated as the mean of three voluntary maximum isometric contractions separated by 1 minute intervals.

**Assays**

We measured dehydroepiandrosterone sulphate (DHEAS), $\alpha$-4-androstendione (A), testosterone (T), dihydrotestosterone (DHT) and 17$\beta$-estradiol by an in-house radioimmunoassay after extraction and subsequent celite chromatography (18). We estimated free testosterone (fT) by a method described by Bartsch, based on measurement of 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 (19). We analyzed sex hormone-binding globulin (SHBG) by double monoclonal immunofluorometric assay (AutoDelfia, Wallac OY,
Finland); intra- and inter-assay CV was 7.5 and 5.2%, respectively. Our inter- and intra-assay 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%; 17β-estradiol, 10.5%, 7.4%. Plasma intact PTH was measured by a chemiluminescence assay using an automated instrument (Immufite, DPC, Los Angeles, CA). Urine N-terminal cross-linking telopeptide of type I collagen (NTX) was measured by an immunometric assay using a Vitros ECI analyser (Ortho Clinical Products, Amersham Pharmacia Biotech, Little Chalfont, UK). This assay uses monoclonal antibodies against human NTX (20). Plasma osteocalcin (total OC) was measured using the N-Mid-Osteocalcin assay on an automated analyser (Elecsys 2010 analyzer, Roche Diagnostics, Mannheim, Germany) with antibodies that determine both intact OC and the large N-Mid terminal fragment. The coefficient of variation (CV) was 4–7% for the various automated assays. Plasma bone-specific alkaline phosphatase (bone ALP) was measured after lectin precipitation using a Hitachi 917 automated analyzer (Roche Diagnostics) (21). This assay was performed with a CV of 8%. Plasma concentrations of procollagen I N-terminal propeptide (PINP) (22), procollagen III N-terminal propeptide (PIIINP) (23), and C-terminal cross-linking telopeptide of type I collagen (ICTP) (24) were determined by commercial RIA kits (Orion Diagnostica, Espoo, Finland). Intra- and interassay CV of 5 and 7%, respectively, were observed. As indices of the IGF axis, serum IGF-I and IGF-II were measured by noncompetitive time-resolved immunofluorometric assays (25), serum IGFBP-2 was measured by RIA, and IGFBP-3 was measured by an immunoradiometric assay (Diagnostics Systems Laboratories, Inc., Webster, TX). Insulin was determined by a commercial ELISA (Dako, Glostrup, Denmark). Leptin was determined by a commercial radioimmunoassay (Linco, St. Louis, MO). Cortisol was measured by an automated chemiluminescence system (Chiron Diagnostics, Fernwald, Germany). Free fatty acids (FFAs) were determined by a colorimetric method using a commercial kit (Wako Chemicals, Neuss, Germany). Total concentrations of T₄ and T₃ were
measured in serum as previously described (26), whereas TSH was measured in a solid-phase, two-site chemiluminescent enzyme immunometric assay (Immulite; Diagnostic Products Corp., Los Angeles, CA). We measured serum total adiponectin by a novel in-house time-resolved immunofluorometric assay (TR-IFMA). Urea excretion in urine was determined by an indophenol method and serum urea by a commercial kit (COBAS INTEGRA; Roche, Hvidovre, Denmark).

Safety parameters

Standard blood count, liver enzymes, electrolytes and renal parameters were measured 4 times during each treatment period and analyzed with routine methods in hospital central laboratory. There were no significant changes and no remarkable alterations.

Statistical analysis

All statistical calculations were carried out using SPSS for Windows version 13.0 (SPSS, Inc., Chicago, Illinois, USA). Data were examined by Student’s two-tailed unpaired t-test or the Mann–Whitney U-test, where appropriate. All data were tested for period as well as carryover effects, which did not affect the level of significance. Analysis was done on delta values (“end period value” minus “start period value”). $P < 0.05$ was considered statistically significant.
Results:

Levels of DHEA, DHEAS, A, T, fT rose significantly to medium normal fertile range during active treatment compared with baseline. No significant alterations were detected in E2 and SHBG values (table 1).

Anthropometric data (weight (kg) 71.3±4.3; BMI (kg/m^2) 25.7±1.7; waist-hip ratio 0.81±0.01) did not change significantly during the treatment period. Lean body mass (LBM) increased during active treatment (delta LBM (kg) placebo vs. DHEA: -0.48±6.1 vs. 1.6±3.4, p=0.02). There was no change in total fat mass (delta TFM (kg), placebo vs. DHEA: -1.2±0.6 vs. 0.6±0.9, p=0.1). We observed no change in abdominal fat distribution measured by CT scan in total abdominal area, subcutaneous fat area or intra-abdominal fat area (figure 1).

Energy expenditure and estimated oxidation rates of glucose (p=0.4), lipid (p=0.6) and protein (p=0.6), as well as the respiratory quotient did not change during the study (figure 2). No changes were seen in thyroid hormones, IGF-I, IGF-II, IGFBP-3, GHPB, free fatty acids, leptin or adiponectin (table 2). Insulin levels did not respond differently to DHEA treatment and substituted cortisol levels were stable throughout the study (data not shown).

Total and regional bone mineral content (BMC) did not increase in response to DHEA treatment (figure 3). A slight, but significant increase in PTH was detected during DHEA compared to placebo treatment (figure 4), however, this change was not followed by any changes in levels of calcium or alkaline phosphatase or indicators of resorptive and formative bone cell activity (figure 4).

The mRNA expression of the investigated inflammatory markers (adiponectin, IL-6, IL-10, MCP-1, and TNF-α) in subcutaneous abdominal fat and in skeletal muscle did not change significantly in response to DHEA treatment, nor did the expression of 11β-HSD mRNA (table 3).
Muscle strength of right arm elbow flexion (placebo start – end vs. DHEA start – end; 182±5 – 189±9 vs. 188±8 – 189±6 Newton meters (Nm), p=0.6) and right leg knee extension (362±26 – 375±26 vs 346±26 – 385±20 Nm, p=0.5) and maximal oxygen consumption (VO2-max (ml/kg/min): 31.1±2.5 – 30.9±3.0 vs. 32.7±2.0 – 31.3±2.2, p=0.5) did not change during the study.

Six patients experienced side effects during active treatment only and one patient reported mild facial greasiness after four days of placebo treatment. Side effects were self reported and were not evaluated by physical examination. Two patients reported symptoms from the scalp region after 64 days and 15 days of treatment. Symptoms were described as “painful spots” and “an unpleasant crawling sensation”, respectively. Both patients left the study. Three patients reported facial greasiness after 29, 36 and 46 days of active treatment and 1 patient experienced regain of axils and pubic hair after 161 days of active treatment.
Discussion

In this randomised, placebo-controlled trial six months of DHEA substitution in women with adrenocortical insufficiency was associated with a complete biochemical normalisation of circulating androgen levels. The only significant effect was a small gain in LBM of 1.6 kg. This occurred without concomitant changes in body weight or fat mass. All other examined variables were essentially unchanged including both subcutaneous and intra-abdominal fat mass determined by CT scan, and we found no alterations in fuel metabolism or in the expression of mRNA of a host of inflammatory markers.

DHEA is regarded an androgen precursor with no affinity for the androgen receptor but with in vitro effects on several membrane-associated receptors (27), including direct binding to estrogen receptors in the growth plate (28). The downstream conversion of DHEA to testosterone and DHT, as well as aromatisation to estrogen, may take place inside target cells. A local degradation of the receptor-bound hormone would then obscure a sex hormone action from serological detection, hence the term “intracrinology” has been introduced (29). Effects of DHEA treatment in adrenocortical failure beyond 12 month have not been evaluated, and in agreement with our findings one year of DHEA treatment increased total lean body mass by 1.8%, with the increase being seen in females only (14). In the same study an increase in BMD at the femoral neck was seen only in DHEA treated patients. In contrast, no significant alterations were seen on DXA scans of body composition and BMD in 39 male and female patients after 3 months (10) and in 38 female hypopituitary women after 6 months (11). Like in the present study, no changes in circulating bone markers have been reported in either mixed female and male populations (10), or in female patients only (11;13;30). In the present study we also evaluated muscle function, determined as muscle strength, and found absolutely no change in this measure, and likewise we did not detect any change in maximal oxygen uptake, another measure which reflects the size of the muscle mass.
In spite of some evidence of minor lean body mass accumulation in response to DHEA substitution, we (31) and others (32) have not found any alterations in forearm and whole body amino acid turnover in short term trials (one and two weeks). In accordance with our previous short term study on growth hormone secretory patterns (8), we did not find any augmentation of growth hormone derived growth factors or their binding proteins. Slightly stimulated IGF-I levels are reported in some (9;14;15) but not all studies (8;12;13;33). In a 3 months study with DHEA or placebo treatment (50 mg) to a group of middle aged female Addison patients insulin sensitivity increased (33), while others found unchanged insulin sensitivity (11;31). In the present study we did not find any evidence of altered fuel metabolism by indirect calorimetry or any changes in pertinent hormones and metabolites. As expected, the surrogate markers of adipose tissue mass, adiponectin and leptin, were unaffected by DHEA treatment. In line with these findings, mRNA expression of specific inflammatory markers in both fat tissue and skeletal muscle remained unchanged. As mentioned above, we did find a slight increase in LBM, but without concomitant changes in any other parameters. However, LBM and other DXA derived measures) are determined with a precision of about 2%. The observed difference in LBM could not be replicated in relation to body weight (determined with a precision (or imprecision) of up to 3 kg (about 5%)), or supported by any other measures of body composition, anthropometrics, serum samples or fat and muscle samples, why we rather consider the finding as a chance finding.

We detected a high proportion (60%) of self reported side effects during active treatment. All reports related to skin problems and probably reflect stimulation of the pilosebaceous unit. Our findings are consistent with the frequency reported in previous trials (9-14) and it may be concluded that the skin is the most DHEA sensitive organ. However, attempts to evaluate this phenomenon with sebutape (14) and body hair score (11) have failed.
The design of the present pilot study, however, has limitations. Only 8 patients completed the trial, which introduces a risk for a type 2 error. It is also possible that longer term duration of treatment would show (positive) changes in body composition, although we doubt this, due to the almost complete absence of the positive effects after 6 month treatment. In addition, the expected effect size of the measured variables may also preclude definite conclusions. However, under similar conditions using small study samples significant effects on body composition has been found. For instance, using the same methodology in our own laboratory, we observed profound changes in body composition in females with Turner syndrome (n=12) treated with growth hormone or placebo after only 2 months of treatment (34). In addition, a study of postmenopausal women treated with a weak androgen (nandrolone) 10 females increased their muscle mass and decreased fatmass in comparison with 10 females receiving placebo and 10 females receiving an anti-androgen (spironolactone) (35). Thus, exclusion of a physiological effect of DHEA may not be possible based on 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.

In conclusion, the present evaluation of DHEA treatment in female adrenocortical failure does not document convincing anabolic effects and does not contribute to a scientific justification for this treatment modality.

Acknowledgement

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**Declaration of interest**

We declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.
Reference List


Legend figure 1:
Intra abdominal and subcutaneous fat tissue area estimated by computed tomography at umbilical level in transversal section view, in female adrenocotical failure before and after 6 months treatment with 50 mg DHEA/placebo.

Legend figure 2:
Resting energy expenditure and estimated fractions of glucose (black), lipid (bright) and protein (gray) oxidation from indirect calorimetry in female adrenocortical failure before and after 6 months treatment with 50 mg DHEA/placebo.

Legend figure 3:
Changes in bone mineral content (BMC) in spine and hip in female patients with adrenocortical failure before and after 6 months treatment with 50 mg DHEA/placebo (expressed as delta changes).

Figure 4: Serum levels of markers of bone metabolism (PTH and Calcium), bone resorption (PINP, PIIINP, ITCP and PICP) and bone formation (alkaline phosphatase and osteocalcin) in female patients with adrenocortical failure before and after 6 months treatment with 50 mg DHEA or placebo.
<table>
<thead>
<tr>
<th>Table 1</th>
<th>Normal range (fertile)</th>
<th>Placebo Start</th>
<th>Placebo End</th>
<th>DHEA Start</th>
<th>DHEA End</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHEA (nmol/l)</td>
<td>3.8 - 15.9</td>
<td>1.74 (1.57 - 2.59)</td>
<td>2.25 (1. - 3.92)</td>
<td>2.11 (0.47 - 3.97)</td>
<td>10.28 (5.13 - 17.17)</td>
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<tr>
<td>DHEAS (nmol/l)</td>
<td>1200 – 9500</td>
<td>99 (99 - 240)</td>
<td>99 (99 - 210)</td>
<td>99 (99 - 230)</td>
<td>8450 (1000 - 19000)</td>
<td>0.012</td>
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<tr>
<td>Androstendione (nmol/l)</td>
<td>2.4 - 8.9</td>
<td>1.9 (0.27 - 3.91)</td>
<td>2.1 (0.21 - 3.75)</td>
<td>2.0 (0.18 - 5.15)</td>
<td>5.1 (1.89 - 9.77)</td>
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<tr>
<td>DHT (nmol/l)</td>
<td>0.25 - 1.2</td>
<td>0.31 (0.09 - 0.45)</td>
<td>0.27 (0.09 - 0.35)</td>
<td>0.28 (0.09 - 0.45)</td>
<td>0.83 (0.38 - 2.02)</td>
<td>0.012</td>
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<tr>
<td>Testosterone (nmol/l)</td>
<td>0.55 - 1.8</td>
<td>0.55 (0.2 - 0.71)</td>
<td>0.52 (0.18 - 0.96)</td>
<td>0.49 (0.16 - 1.5)</td>
<td>1.30 (0.69 - 2.65)</td>
<td>0.012</td>
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<tr>
<td>Free Testosterone (nmol/l)</td>
<td>0.006 - 0.034</td>
<td>0.01 (0 - 0.01)</td>
<td>0.01 (0 - 0.01)</td>
<td>0.00 (0 - 0.02)</td>
<td>0.02 (0.01 - 0.03)</td>
<td>0.018</td>
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<tr>
<td>E2 (pmol/l)</td>
<td>&lt;40 – 400</td>
<td>155 (39 - 540)</td>
<td>195 (39 - 1100)</td>
<td>290 (39 - 630)</td>
<td>220 (39 - 630)</td>
<td>0.3</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>41 – 170</td>
<td>88 (27 - 114)</td>
<td>96 (37 - 156)</td>
<td>78 (36 - 163)</td>
<td>79 (26 - 144)</td>
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</table>

Legend table 1:

Serum values (median, range) of androgens and androgen precursor metabolites in female adrenocortical failure before and after 6 months treatment with 50 mg DHEA or placebo.
Table 2

<table>
<thead>
<tr>
<th></th>
<th>placebo start</th>
<th>placebo end</th>
<th>DHEA start</th>
<th>DHEA end</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol (nmol/L)</td>
<td>640 ± 77</td>
<td>463 ± 142</td>
<td>475 ± 89</td>
<td>492 ± 142</td>
<td>0.3</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>97 ± 45</td>
<td>81 ± 16</td>
<td>47 ± 7</td>
<td>54 ± 6</td>
<td>0.7</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>17.0 ± 2.2</td>
<td>13.5 ± 2.1</td>
<td>14.5 ± 2.8</td>
<td>15.0 ± 2.2</td>
<td>0.2</td>
</tr>
<tr>
<td>T3 (nmol/L)</td>
<td>1.7 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>T4 (nmol/L)</td>
<td>93 ± 6</td>
<td>89 ± 7</td>
<td>89 ± 7</td>
<td>91 ± 9</td>
<td>0.6</td>
</tr>
<tr>
<td>TSH (µU/ml)</td>
<td>2.0 ± 0.6</td>
<td>2.3 ± 0.6</td>
<td>2.7 ± 1.0</td>
<td>2.6 ± 0.5</td>
<td>0.9</td>
</tr>
<tr>
<td>GHBP (nmol/L)</td>
<td>2.4 ± 0.2</td>
<td>2.7 ± 0.3</td>
<td>2.5 ± 0.3</td>
<td>2.2 ± 0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>IGF-1 (ug/L)</td>
<td>212 ± 26</td>
<td>201 ± 17</td>
<td>212 ± 27</td>
<td>213 ± 15</td>
<td>0.5</td>
</tr>
<tr>
<td>IGF-2 (ug/L)</td>
<td>796 ± 50</td>
<td>800 ± 46</td>
<td>751 ± 40</td>
<td>798 ± 56</td>
<td>0.1</td>
</tr>
<tr>
<td>IGFBP3 (ug/L)</td>
<td>4853 ± 261</td>
<td>4986 ± 320</td>
<td>4823 ± 370</td>
<td>5039 ± 402</td>
<td>0.7</td>
</tr>
<tr>
<td>FFA (mmol/L)</td>
<td>0.38 ± 0.05</td>
<td>0.40 ± 0.10</td>
<td>0.48 ± 0.07</td>
<td>0.35 ± 0.07</td>
<td>0.2</td>
</tr>
<tr>
<td>Adiponectin (mg/l)</td>
<td>14.9 ± 2.3</td>
<td>14.0 ± 2.4</td>
<td>15.8 ± 2.9</td>
<td>14.9 ± 2.6</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Legend table 2:

Serum values (mean and standard error of the mean) of hormones, fat tissue markers and free fatty acids in female adrenocortical failure before and after 6 months treatment with 50 mg DHEA or placebo.
Table 3:

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>DHEA</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fat</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.007 ± 0.002</td>
<td>0.008 ± 0.003</td>
<td>0.8</td>
</tr>
<tr>
<td>MCP-1</td>
<td>0.002 ± 0.001</td>
<td>0.006 ± 0.003</td>
<td>0.2</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.023 ± 0.010</td>
<td>0.032 ± 0.018</td>
<td>0.5</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.008 ± 0.002</td>
<td>0.019 ± 0.009</td>
<td>0.2</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>10.0 ± 3.6</td>
<td>10.0 ± 2.5</td>
<td>1.0</td>
</tr>
<tr>
<td>11β-HSD</td>
<td>0.308 ± 0.128</td>
<td>0.497 ± 0.209</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Muscle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.028 ± 0.023</td>
<td>0.008 ± 0.002</td>
<td>0.4</td>
</tr>
<tr>
<td>MCP-1</td>
<td>0.006 ± 0.005</td>
<td>0.001 ± 0.000</td>
<td>0.3</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.130 ± 0.105</td>
<td>0.028 ± 0.006</td>
<td>0.4</td>
</tr>
</tbody>
</table>

mRNA expression (relative gene expression of target gene to β-actin) of adipokines in subcutaneous abdominal fat and in muscle tissue in female adrenocortical failure after 6 months treatment with placebo or DHEA.
Figure 1

CT abdominal area, transversal section view

Total Abdominal Area

Subcutaneous Fat

Intra Abdominal Fat

p=0.5

p=0.6

p=1.0

Placebo  DHEA  Placebo  DHEA  Placebo  DHEA

Total Abdominal Area

Subcutaneous Fat

Intra Abdominal Fat

p=0.5

p=0.6

p=1.0

Placebo  DHEA  Placebo  DHEA  Placebo  DHEA
Figure 2

Indirect Calorimetry

- kcal/24h
- Placebo
- DHEA
- p=0.5
- Start
- End
- Start
- End

Graph showing indirect calorimetry results with Placebo and DHEA treatments, indicating no significant difference (p=0.5) between the start and end phases for both groups.
Figure 3

![Graph showing ΔBMC spine and ΔBMC hip with Placebo and DHEA groups.](image)

- ΔBMC spine: P=0.2
- ΔBMC hip: P=0.4
Figure 4.