

Dehydroepiandrosterone Replacement Therapy in Hypoadrenal Women: Protein Anabolism and Skeletal Muscle Function

KETAN K. DHATARIYA, MSc, MD, FRCP; LAURA J. S. GREENLUND, MD, PhD; MAUREEN L. BIGELOW, RN; PRABIN THAPA, MS; ANN L. OBERG, PhD; G. CHARLES FORD, MSc; JILL M. SCHIMKE, MSc; AND K. SREEKUMARAN NAIR, MD, PhD

OBJECTIVE: To determine whether dehydroepiandrosterone (DHEA) replacement therapy in hypoadrenal women improves performance, muscle protein accretion, and mitochondrial functions.

PARTICIPANTS AND METHODS: Thirty-three hypoadrenal women were enrolled in the study from May 1, 2002, through May 31, 2003. Twenty-eight completed a 12-week, prospective, randomized, placebo-controlled, crossover study with either daily placebo or 50 mg of DHEA with a 2-week washout period and then crossed over to the other treatment. Body composition, physical performance, whole-body and muscle protein metabolism, and mitochondrial functions were determined.

RESULTS: Administration of DHEA significantly increased plasma levels of DHEA sulfate, testosterone, and androstenedione but did not change body composition, muscle strength, peak aerobic capacity, and whole-body protein turnover or synthesis rates of mitochondrial, sarcoplasmic, or mixed muscle proteins. Muscle mitochondrial oxidative enzymes and messenger RNA (mRNA) levels of genes encoding mitochondrial proteins and nuclear transcription factors did not change after DHEA administration. However, mRNA levels of muscle myosin heavy chain 1 ($P=.004$), which determines muscle fiber type, and those of insulinlike growth factor binding proteins 4 and 5 significantly decreased ($P=.02$ and $P=.03$, respectively).

CONCLUSION: Three months of DHEA administration increased DHEA sulfate and androgen levels but had no effect on physical performance, body composition, protein metabolism, or muscle mitochondrial biogenesis in hypoadrenal women. However, lowering of mRNA levels of binding proteins of insulinlike growth factor 1 and myosin heavy chain 1 suggests potential effects of long-term treatment with DHEA on muscle fiber type.

Trial Registration: clinicaltrials.gov identifier: NCT00279929

Mayo Clin Proc. 2008;83(11):1218-1225

COX = cytochrome c oxidase; DHEA = dehydroepiandrosterone; DHEA-S = DHEA sulfate; FFM = fat-free mass; FSR = fractional synthesis rate; $[^2\text{H}_4]$ = [ring 2,3,5,6- $^2\text{H}_4$]; IGF = insulinlike growth factor; IGFBP = IGF binding protein; MYH = myosin heavy chain; mRNA = messenger RNA; NADH4 = nicotinamide adenine dinucleotide phosphate 4; $[^{15}\text{N}]$ = nitrogen 15; PCR = polymerase chain reaction; PPARGC1A = peroxisome proliferator-activated receptor- γ coactivator 1 α ; TFAM = transcription factor A, mitochondrial; $\dot{V}_{O_{2peak}}$ = peak oxygen consumption

From the Endocrine Research Unit (K.K.D., M.L.B., G.C.F., J.M.S., K.S.N.), and Division of Biostatistics (P.T., A.L.O.), Mayo Clinic, Rochester, MN; and Innovations Biomedical Consulting (L.J.S.G.), Rochester, MN. Dr Dhatariya is now at the Elsie Bertram Diabetes Centre, Norfolk and Norwich University Hospital NHS Trust, Norfolk, UK.

This work was supported by National Institutes of Health grants UL1 RR024150-01, P01AG14383-05, and R01AG09531.

Individual reprints of this article are not available. Address correspondence to K. Sreekumaran Nair, MD, PhD, Endocrine Research Unit, Mayo Clinic, 200 First St SW, Rochester, MN 55905 (nair@mayo.edu).

© 2008 Mayo Foundation for Medical Education and Research

Adrenally produced dehydroepiandrosterone (DHEA) and its sulfated ester (DHEA-S) are the most abundant steroid hormones found in the circulation. These hormones peak during the late teenage years and steadily decrease by 10% per decade thereafter.¹⁻⁴ As a result, healthy elderly men and women have lower DHEA and DHEA-S levels than younger healthy individuals. Levels are low or almost undetectable in women with primary hypoadrenalism due to Addison disease or in those who have undergone bilateral adrenalectomies. Although DHEA is not required for survival, it is known to be a precursor of sex hormones and is converted into androgens and estrogens in peripheral tissues.⁵ Its other physiologic functions in healthy adults remain unclear. A recent review of the epidemiological associations and effects of replacement of DHEA on body composition, bone density, and muscle function has shown conflicting reports.⁶

Studies have examined the effect of DHEA replacement on skeletal muscle strength and lean body mass in patients with low DHEA levels, including those who are hypoadrenal and healthy elderly people.^{7,8} The study of elderly people with low DHEA-S levels showed no improvement of physical performance, body composition, insulin sensitivity, or bone density.⁸ However, the studies of elderly patients cannot be translated to hypoadrenal patients, who have markedly lower DHEA-S levels. Levels of DHEA-S decrease with age, reducing skeletal muscle oxidative capacity, muscle protein synthesis, and exercise performance.^{9,10} Whether DHEA replacement improves these muscle functions in hypoadrenal women with low DHEA-S levels remains to be determined.

The low or absent levels of DHEA or DHEA-S found in women who have Addison disease or who have undergone adrenalectomy may contribute to the decline in their muscle mass and function. Although short-term resistance exercise programs significantly improve skeletal muscle mass,¹¹ small changes in muscle mass after short-term therapy with DHEA may not have been detected and so not reported in previous studies. Measurement of muscle protein synthesis responds more intensely to short-term intervention and may be a better method of detecting these changes.¹² Recent studies of DHEA replacement in an Addisonian population showed an increase in truncal lean body mass,¹³ and other

investigators showed that DHEA given to a healthy elderly population led to increasing strength after weight training¹⁴; however, no detailed explanation for possible mechanisms was offered. The current study measured muscle protein synthesis rates to determine whether DHEA replacement affected skeletal muscle protein synthesis rates, accounting for this anabolic effect. In elderly people, muscle mitochondrial function declines as levels of DHEA decrease. However, relatively short-term aerobic exercise programs improved mitochondrial oxidative capacity in elderly people.¹⁵ In the current study, we assessed whether administration of DHEA for 3 months to DHEA-deficient patients would improve mitochondrial biogenesis.

Muscle functions and performance depend on mitochondrial function and the composition of contractile proteins, such as the isoforms of the myosin heavy chain (MYH). We therefore determined whether DHEA replacement therapy increases levels of muscle mitochondrial enzymes, mitochondrial-related proteins, and transcription factors, as well as levels of MYH isoforms in messenger RNA (mRNA) and fractional synthesis rates (FSRs) of mitochondrial and sarcoplasmic fraction of muscle proteins.

The aim of our study was to test the hypothesis that 12 weeks of DHEA replacement therapy in hypoadrenal women with profoundly low levels of DHEA would improve muscle strength and muscle metabolic processes. These women were also prescribed a standardized glucocorticoid replacement regimen.

PARTICIPANTS AND METHODS

This single-center study had a randomized, placebo-controlled, crossover design. The study protocol and consent form were approved by the Mayo Clinic Institutional Review Board. All experiments conducted conformed to the standards set by the latest revision of the Declaration of Helsinki. Written informed consent was obtained from all volunteers. Participant characteristics and inclusion and exclusion criteria have been previously described in an article showing that DHEA replacement therapy increased insulin sensitivity.¹⁶

Of the 33 hypoadrenal women randomized from May 1, 2002, through May 31, 2003, 28 (85%) completed the study. The 5 remaining participants withdrew for the following reasons: 3 withdrew without completing the first arm of the study (1 because of diarrhea, 1 because she unblinded herself, and 1 because of pressure at work; all while taking DHEA), and 2 withdrew after completing the first arm of the study (both while taking placebo).

The mean \pm SD age of the 28 study participants who completed the study was 50.3 ± 15.9 years, and the mean \pm SD body mass index (calculated as the weight in kilograms

divided by height in meters squared) was 27.0 ± 4.4 . Fourteen women were postmenopausal, with a mean \pm SD length of time being hypoadrenal of 12.3 ± 1.9 years. Twenty women (71%) had primary Addison disease and other diagnoses, including bilateral adrenalectomy due to Cushing syndrome (n=5), benign bilateral pheochromocytomas (n=1), and congenital adrenal hyperplasia (n=1).

The same women received 12 weeks each of both placebo and DHEA. A total of 50 mg of pharmaceutical-grade microencapsulated DHEA (Medisca, Plattsburgh, NY) or identically encapsulated placebo containing lactose (Clinical Encapsulation Services, Schenectady, NY) was self-administered as a single daily dose for 12 weeks each with a 2-week washout period between treatment phases. The chemical purity of DHEA (95% pure on analysis) was tested before preparing capsules.

At screening, routine hematology and biochemistry tests were conducted to assess general health. Further blood tests included measurement of levels of DHEA-S, androstenedione, and bioavailable testosterone. Before the first phase of the study, participants underwent a standard submaximal treadmill stress test to exclude cardiorespiratory abnormalities. All participants received hydrocortisone given in divided doses (mean \pm SD total daily dose, 24.9 ± 7.1 mg) to minimize the potentially confounding effects of different glucocorticoid replacement regimens. Eight participants had previously been taking prednisone (mean \pm SD daily dose, 4.5 ± 1.4 mg). At the end of the first period of drug administration, the participants were admitted to the General Clinical Research Center (currently called the Clinical Research Unit of the Mayo Clinic Center for Translational Science Activities). Participants had indirect calorimetry measured for 30 minutes. They then underwent peak oxygen consumption ($\dot{V}O_{2peak}$) testing by using a stationary bicycle.¹⁷ Participants performed upper and lower body strength tests. Participants were asked to perform a series of 1 to 3 repetitions of seated chest press, bicep curl, knee extension, and leg curl, at higher resistance loads, until the 1-repetition maximum for each participant was determined. Static strength test was assessed by peak handgrip. At the end of the 12-week period, there was a 2-week washout period followed by administration of the alternative preparation for 12 weeks. Participants were studied in an identical fashion to the previous visit.

TRACER INFUSION

Insulin infusion has previously been shown to increase the synthesis rates of skeletal muscle mitochondrial proteins in healthy volunteers.¹⁸ For this reason, as well as to assess insulin sensitivity,¹⁶ a hyperinsulinemic (1.5 IU/kg of fat-free mass [FFM] per minute) euglycemic clamp was used in the current study.^{19,20}

Of the 28 women, 7 (25%) were studied with additional infusions of nitrogen 15 [¹⁵N]-labeled phenylalanine and [ring 2,3,5,6-²H₄] ([²H₄]) tyrosine to measure amino acid kinetics and FSRs of muscle mitochondrial proteins and sarcoplasmic protein subfractions. In the women in whom amino acids were infused, priming doses of [¹⁵N] phenylalanine at 1.6 mg/kg of FFM, [¹⁵N] tyrosine at 0.6 mg/kg of FFM, and [²H₄] tyrosine at 0.6 mg/kg of FFM were given at 4:00 AM. Infusions of [¹⁵N] phenylalanine and [²H₄] tyrosine were then continued at 1.6 mg/kg of FFM per hour and 0.6 mg/kg of FFM per hour, respectively. Infusions lasted for 10 hours, with skeletal muscle needle biopsy specimens being taken at 3 hours and 10 hours after the start of the tracer infusions.

MUSCLE BIOPSIES

With the patient under local anesthesia, vastus lateralis muscle samples (approximately 300 mg each) were obtained using a percutaneous needle as previously described.^{21,22} The first biopsy was performed before the start of the insulin infusion at 7:00 AM. The second biopsy specimen was taken from the contralateral aspect of the thigh after 7 hours.

WHOLE-BODY PROTEIN TURNOVER AND MUSCLE PROTEIN SYNTHESIS

Plasma isotopic enrichment of [¹⁵N] phenylalanine, [¹⁵N] tyrosine, and [²H₄] tyrosine levels was measured using a gas chromatograph/mass spectrometer as previously described.^{23,24} Calculation of phenylalanine flux, tyrosine flux, phenylalanine conversion to tyrosine, and phenylalanine incorporation into protein (protein synthesis) was also performed as previously described.^{23,24} A 150-mg portion of each muscle sample was used for the isolation of mitochondrial and sarcoplasmic protein fractions by differential centrifugation.^{22,25-27} Isotopic enrichments of [¹⁵N] phenylalanine in muscle proteins were determined using gas chromatography-combustion-isotope ratio mass spectrometry (Finnigan MAT, Delta Plus; Finnigan Corporation, San Jose, CA).²⁸ Muscle tissue fluid tracer enrichment and plasma amino acid enrichment were measured as previously described.²⁹

The FSR of mixed muscle protein and mitochondrial and sarcoplasmic proteins was calculated using the following equation³⁰: $FSR (\%/h) = 100 \times (E_{10h} - E_{3h}) / (E_{TF} \times t)$, where $(E_{10h} - E_{3h})$ represents the increment in amino acid enrichment in muscle protein between 3 and 10 hours of infusion, E_{TF} is the average enrichment of amino acids in muscle tissue fluid taken from the 3- and 10-hour biopsy specimens, and t is the time of incorporation between the 2 biopsies (in this study, 7.0 hours; mean, 6.9 hours; 95% confidence interval, 6.8-7.0 hours).

MITOCHONDRIAL ENZYME ACTIVITIES

Maximal enzyme activities of citrate synthase and cytochrome c oxidase (COX) were measured in the tissue homogenate and in the mitochondrial pellet using photospectrometric analyses as previously described.^{18,26} Protein concentrations in the tissue homogenate and the mitochondrial pellet were measured using a commercial kit (Detergent Compatible Protein Assay; Bio-Rad, Hercules, CA).

METABOLITE CONCENTRATIONS

Plasma levels of amino acids were measured with a high-performance liquid chromatography system (HP 1090, 1046 fluorescence detector and cooling system; Hewlett-Packard, Palo Alto, CA).³¹ Glucose was analyzed on site using an enzymatic technique (Beckman Instruments, Fullerton, CA).

HORMONAL ASSAYS

Free and total insulinlike growth factor (IGF) 1, IGF binding protein (IGFBP) 1, IGFBP3, and sex hormone-binding globulin were measured using a 2-site immunoradiometric assay. Levels of DHEA-S were measured using a competitive radioimmunoassay; androstenedione was measured by direct radioimmunoassay. These assays were from Diagnostic Systems Laboratories (Webster, TX). Testosterone was measured with the Coat-A-Count radioimmunoassay (DPC, Los Angeles, CA).

MESSENGER RNA QUANTIFICATION

Messenger RNA extractions were performed on frozen muscle samples from individual study participants as previously described.^{11,19,32} A real-time quantitative polymerase chain reaction (PCR) system (PE Biosystems, Foster City, CA) was used to measure MYH1; MYH2; transcription factor A, mitochondrial (TFAM); peroxisome proliferator-activated receptor- γ coactivator 1 α (PPARGC1A); COX subunits 3 and 4; and reduced nicotinamide adenine dinucleotide phosphate 4 (NADH4) mRNAs. The signal for the 28S ribosomal RNA was used to normalize against differences in RNA isolation and RNA degradation and in the efficiencies of the reverse transcription and PCRs. The final quantitation was achieved with a relative standard curve.¹¹ The sequences for MYH oligos¹¹ and TFAMs^{32,33} have been previously published.

STATISTICAL ANALYSES

Randomization of treatment orders assigned to study participants was performed independently by the Division of Biostatistics at Mayo Clinic. Because this was a crossover study, participants acted as their own controls. The data presented in this article were part of a larger study that also examined the effects of DHEA replacement on the mood, memory, and sexual well-being of hypoadrenal women.

TABLE 1. Effect of Dehydroepiandrosterone (DHEA) Replacement Therapy on Body Composition and Muscle Strength^a

Body composition and bone density	Placebo	DHEA	<i>P</i> value (crossover design)	<i>P</i> value (paired <i>t</i> test)	80% Power to detect difference ^b
Fat (%)	45.4±5.8 ^c	44.8±6.5	.25	.24	1.68
Fat-free mass (kg)	39.7±4.7	39.8±4.6	.60	.61	0.78
Bone density (g/cm ²)	1.2±0.1	1.2±0.1	.69	.63	0.01
Handgrip strength (kg)	29.3±5.9 ^c	29.1±5.9	.70	.75	1.58
Bicep curl (kg) ^d	37.5±6.9 ^c	37.7±7.8 ^c	.89	.69	1.59
Leg press (kg)	57.3±15.9	57.4±14.8	.98	.97	3.19
Chest press (kg)	40.0±8.6 ^c	40.5±9.1 ^c	.59	.47	1.81
Leg curl (kg) ^d	39.6±11.3 ^c	39.8±11.3 ^c	.63	.70	1.86

^a Data are reported as mean ± SD. Unless otherwise stated, n=28.

^b Denotes a percentage change in level.

^c n=27.

^d One study participant was unable to lie down for the biceps curl and was thus not included in that analysis. During the assessment visit for 1 of the participants, the leg curl machine was out of order, and so her data were not included in the analysis.

The power calculation for the larger overall study was based on previous data using the visual analog scale for sexuality assessment used by Arlt et al.³⁴ They showed that changes in components of the scales of sexual activity had SDs ranging from 23.8 to 29.9. On the basis of this, paired *t* tests at the 5% level have 80% power to detect changes of 13.9 to 19.0 or 40.3% to 50.7% of baseline values with 25 individuals. Arlt et al³⁴ observed within-participant changes of this magnitude in individuals receiving DHEA treatment.

Changes in skeletal muscle physiologic function were assessed after 12 weeks of placebo and 12 weeks of DHEA. Paired *t* test and Wilcoxon signed rank sum test were used for analyses. Data are given as mean ± SD. Pearson product correlations were used to determine the strength of association for selected variables. Significant effect for all tests was accepted at *P*<.05.

RESULTS

HORMONE LEVELS

The hormonal results have previously been published.¹⁶ In summary, DHEA treatment significantly increased serum

DHEA-S levels. Significant increases were also seen in levels of bioavailable testosterone and androstenedione, whereas the level of sex hormone-binding globulin was reduced by DHEA treatment.

BODY COMPOSITION, STRENGTH TESTS, EXERCISE PERFORMANCE, AND INDIRECT CALORIMETRY TESTS

Dehydroepiandrosterone treatment had no effect on percentage of fat, FFM, bone mineral density, or upper and lower extremity strength (as determined by performance on handgrip, biceps curl, chest press, leg curl, and leg press) (Table 1). Indirect calorimetry, respiratory quotient, and resting energy expenditure showed no changes in response to DHEA treatment. Stationary bike testing of $\dot{V}O_{2peak}$, peak bike power, and heart rate also showed no significant differences between DHEA and placebo. Data are shown in Table 2.

PROTEIN TURNOVER, PROTEIN FSRs, AND MUSCLE mRNA

In whole-body protein turnover, no significant differences were noted for phenylalanine or tyrosine flux; phenylalanine conversion to tyrosine, representing the catabolic fate of phenylalanine; and phenylalanine incorporation into pro-

TABLE 2. Indirect Calorimetry Data and Aerobic Performance^{a,b}

Variable	Placebo	DHEA	<i>P</i> value (crossover design)	<i>P</i> value (paired <i>t</i> test)	80% Power to detect difference ^c
Indirect calorimetry resting $\dot{V}CO_2$ (mL/min)	170.2±20.2	170.5± 20.5	.97	.94	11.05
Indirect calorimetry resting $\dot{V}O_2$ (mL/min)	205.1±27.0	205.0±24.9	.96	.98	10.06
Respiratory quotient	0.8±0.1	0.83±0.06	.95	.95	0.04
Resting energy expenditure (kcal/d)	1421.5±176.4	1418.3±167.2	.85	.90	68.01
Peak bike power (W)	119.8±35.7 ^d	115.9±34.4	.15	.16	5.91
$\dot{V}O_{2peak}$ per kilogram of FFM (mL/min)	36.7±8.4	36.3±7.4	.67	.62	2.03
Peak heart rate (/min)	160.0±21.5 ^e	161.3±21.7	.13	.13	7.25

^a FFM = fat-free mass; $\dot{V}CO_2$ = carbon dioxide consumption; $\dot{V}O_2$ = oxygen consumption per unit time; $\dot{V}O_{2peak}$ = peak oxygen consumption.

^b Data are reported as mean ± SD. Unless otherwise stated, n=28.

^c Denotes a percentage change in level.

^d n=27.

^e n=26.

TABLE 3. Effect of Dehydroepiandrosterone (DHEA) Replacement on Whole-Body Phenylalanine and Tyrosine Flux Values and FSR or Mitochondrial Sarcoplasmic and Mixed Muscle Protein^a

	Placebo	DHEA	<i>P</i> value (paired <i>t</i> test)	<i>P</i> value (crossover design)	80% Power to detect difference ^b
Phenylalanine flux (μmol/kg hourly)	55.4±5.0	52.2±5.8	.07	.43	5.81
Tyrosine flux (μmol/kg hourly)	45.4±4.4	44.6±4.2	.68	.49	5.94
Phenylalanine conversion to tyrosine (μmol/kg hourly)	5.0±1.0	4.6±0.7	.52	.76	2.18
Phenylalanine incorporation to protein (μmol/kg hourly) (protein synthesis)	50.3±4.8	47.6±5.5	.07	.33	5.34
Mitochondrial FSR (%/h)	0.19±0.11	0.16±0.04	.64	.75	0.03
Sarcoplasmic FSR (%/h)	0.12±0.06	0.11±0.03	.77	.71	0.02
Mixed muscle protein FSR (%/h)	0.06±0.02	0.05±0.01	.62	.75	0.03

^a Fractional synthesis rate (FSR) calculations were performed using muscle tissue fluid [¹⁵N] phenylalanine enrichment as precursor pool.²⁹ Data are reported as mean ± SD.

^b Denotes a percentage change in level.

teins, representing protein synthesis (Table 3). The FSRs of sarcoplasmic proteins and mitochondrial proteins are given in Table 3, indicating no differences between the 2 studies. Muscle mRNA levels for TFAM and myosin isoforms were measured by real-time quantitative PCR. In contrast, mRNA levels of PPARGC1A, TFAM, nuclear respiratory factor 1, COX3, COX4, and NADH4 did not change with treatment (data not shown). We also

found no differences in COX levels (89.22±22.48 μmol/kg per hour of protein for the placebo group vs 96.35±13.11 μmol/kg per hour of protein for the DHEA group; *P*=.55) and citrate synthase activity (137.07±39.56 μmol/kg per hour of protein for the placebo group vs 142.11±35.66 μmol/kg per hour of protein for the DHEA group; *P*=.46) in muscle. A significant decrease was seen in MYH1 mRNA in response to DHEA treatment (Figure), but no significant

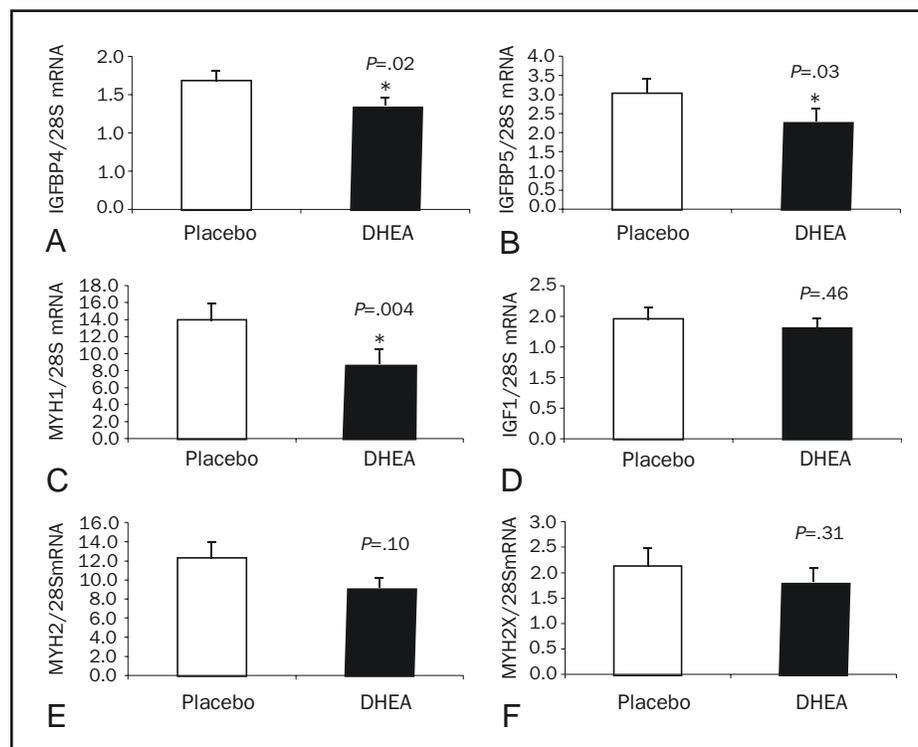


FIGURE. Skeletal muscle messenger RNA (mRNA) levels showing that dehydroepiandrosterone (DHEA) administration significantly decreased insulinlike growth factor binding protein (IGFBP) 4 (A), IGFBP5 (B), and myosin heavy chain (MYH) 1 (C) mRNA levels with no significant effects on those of insulinlike growth factor 1 (IGF1) (D), MYH2 (E), and MYH2X (F). Asterisk indicates statistical significance.

differences were noted for MYH2 and MYH2X. A significant decrease in mRNA levels of IGFBP4 and IGFBP5 was noted, but no changes in IGF1 or androgen receptor were observed.

DISCUSSION

The current study shows that 50 mg of once-daily DHEA administration in hypoadrenal women aged 20 to 80 years for 3 months had no significant effects on energy metabolism, $\dot{V}O_{2peak}$, muscle strength, muscle protein synthesis, or mitochondrial enzymes. However, DHEA replacement reduced skeletal muscle mRNA expression of MYH1, IGFBP4, and IGFBP5 but had no effect on mRNA levels of selected nuclear and mitochondrial genes encoding mitochondrial proteins and transcription factors. After DHEA replacement, DHEA-S levels in these women¹⁶ increased to the high levels that are commonly observed in young people.⁸

Little work has been done on physical performance or skeletal muscle protein synthesis rates in hypoadrenal women. An animal study has shown that adrenalectomy per se does not affect skeletal muscle protein synthesis rates.³⁵ The lack of effect of DHEA on muscle function in our study is in agreement with previous studies performed in elderly people with low DHEA levels who were receiving DHEA replacement therapy.^{8,36} Our findings are also consistent with those of another study of postmenopausal women in whom DHEA administration offered no additional benefits to the exercise program.³⁷ However, they contradict those of a recent study¹⁴ showing that supplemental DHEA administration for 6 months in elderly people modestly enhances resistant exercise-induced increases in muscle mass.

Consistent with the lack of effect on muscle strength and body composition, DHEA replacement did not affect mixed muscle, sarcoplasmic, or mitochondrial protein synthesis. Previous work has shown that testosterone replacement enhances muscle protein synthesis in hypogonadal men,³⁸ but the current study shows that, despite an increase in levels of androgens such as bioavailable testosterone, DHEA replacement did not affect muscle protein synthesis in hypoadrenal women. Muscle protein synthesis was not stimulated by a modest increase in testosterone for 2 possible reasons. First, the effect of testosterone on muscle protein synthesis may be dose dependent, and so higher testosterone levels may have increased muscle protein synthesis. A dose effect of testosterone has been previously reported.^{39,40} Second, testosterone may not affect muscle protein synthesis in women.

The decrease in MYH1 mRNA with DHEA replacement is of great interest because aging, characterized by

DHEA deficiency, is associated with a decrease in MYH2 and MYH2X mRNA expression with no effect on MYH1.^{41,42} In type I muscle fibers, MYH1 protein expression is higher, and MYH1 mRNA expression is higher in older people.^{11,43} Our study shows that MYH1 mRNA expression decreases in muscle with DHEA replacement therapy; however, the functional implications of this observation remain unclear. Because MYH1 is a protein with a slow synthesis rate,⁴² alteration of its composition in muscle and associated functional changes may take longer than mRNA changes.

Our results showing the lack of effect of DHEA on energy expenditure using indirect calorimetry are consistent with previous animal and human data.^{7,44} The lack of any effect of DHEA on energy metabolism at the whole-body level is consistent with the observation in muscle mitochondrial metabolism. Muscle mitochondrial function as assessed by enzyme activities was not affected by DHEA replacement. The enzymes COX and citrate synthase were measured because they form an integral part of the electron transport chain and adenosine triphosphate production. Mitochondrial enzyme activity can be used as a surrogate for DHEA production capacity²⁶; thus, it is unlikely that DHEA affects adenosine triphosphate production. This may account for the lack of change in physical performance seen with $\dot{V}O_{2peak}$ or skeletal muscle strength. Moreover, mRNA levels of mitochondrial proteins encoded by nuclear and mitochondrial genes were not altered by DHEA replacement therapy. The mRNA levels of mitochondrial transcription factors, PPARGC1A, nuclear respiratory factor 1, and TFAM also remained unchanged between the DHEA and placebo arms of the study, helping elucidate an integral part of the mechanism responsible for mitochondrial DNA transcription and replication.⁴⁵ These transcription factors have been shown to be key regulators of exercise-induced mitochondrial gene expression and thus oxidative capacity.⁴⁶ In our study, DHEA replacement therapy affected neither mRNA levels of these nuclear transcription factors nor mitochondrial function.

Of note, although mRNA levels of IGF1 did not change, mRNA levels of IGFBP4 and IGFBP5 decreased. The expression of these proteins has a key role in the biological action of IGF1 in muscle.⁴⁷ Although DHEA administration did not change plasma levels of IGF1 or its binding proteins (IGFBP1 and IGFBP3),¹⁶ the possibility that IGF1 could affect muscle in the long term cannot be excluded.

Our study has some limitations. All our volunteers were white, and most came from a tertiary care referral center; as such, referral bias is a possibility. However, more than a third of our participants (n=10) had never been seen at

Mayo Clinic. It is difficult to know whether systematic differences existed between those who did not respond to our invitations to participate in the study and those who took part. Our study included participants with a wide range of ages, and the results should therefore be applicable to white hypoadrenal women older than 18 years; they are likely representative of the female hypoadrenal population as a whole. Whether these results can be applied to other groups, such as people with low DHEA levels and normally functioning adrenal glands or men, remains open to question. The importance of the current study is that maintenance of DHEA-S levels for 3 months in DHEA-deficient women offered an opportunity to assess the physiologic role of DHEA in women, who, unlike men, have very low androgen levels.

CONCLUSION

The current study shows that 50 mg of DHEA given once daily for 3 months to hypoadrenal white women receiving standardized glucocorticoid replacement therapy had no effect on measures of physical strength, exercise capacity, or skeletal muscle protein synthesis and mitochondrial function. No measurable changes in body composition, protein metabolism, physical performance, or muscle mitochondrial biogenesis were noted with DHEA replacement therapy. However, DHEA administration reduced the mRNA expression of IGFBP4, IGFBP5, and MYH1, indicating potential long-term physiologic and anatomic effects.

In the United States, unlike Europe, DHEA remains freely available over the counter as a "health food supplement." This is despite the fact that DHEA is not a food and does not naturally appear in the human food chain and that no foodstuff can carry out its physiologic role. Although DHEA has been shown to increase insulin sensitivity modestly and alter lipid profile favorably in hypoadrenal women, the results of the current study show no appreciable effects of DHEA on physical performance in these women. Because the study lasted only 3 months, it could not fully determine long-term effects; it did, however, suggest a potential effect of DHEA on muscle fiber type and IGF1 action.

We gratefully acknowledge the expert assistance of Sherrie Hanna, Donna Felmlee-Devine, and Erin Foley of the National Adrenal Disease Foundation, Joan Hoffman of Addison News, and Melanie Wong of the Addison's Disease Support Group for Northern Illinois. We also gratefully acknowledge the support of the Mayo Clinic Center for Translational Science Activities Clinical Research Unit staff and the clinical skills of Jane C. Kahl, Dawn M. Morse, Katherine A. Klaus, Xuan-Mai T. Persson, and Lawrence E. Ward.

REFERENCES

- Orentreich N, Brind JL, Rizer RL, Vogelmann JH. Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. *J Clin Endocrinol Metab.* 1984;59(3):551-555.
- Rosenfeld RS, Rosenberg BJ, Fukushima DK, Hellman L. 24-Hour secretory pattern of dehydroisoandrosterone and dehydroisoandrosterone sulfate. *J Clin Endocrinol Metab.* 1975;40(5):850-855.
- Birkenhäger-Gillesse EG, Derksen J, Lagaay AM. Dehydroepiandrosterone sulphate (DHEAS) in the oldest old, aged 85 and over. *Ann N Y Acad Sci.* 1994;719:543-552.
- Nafziger AN, Bowlin SJ, Jenkins PL, Pearson TA. Longitudinal changes in dehydroepiandrosterone concentrations in men and women. *J Lab Clin Med.* 1998;131(4):316-323.
- Labrie F. Intracrinology. *Mol Cell Endocrinol.* 1991;78(3):C113-C118.
- Dhatariya KK, Nair KS. Dehydroepiandrosterone: is there a role for replacement? *Mayo Clin Proc.* 2003;78(10):1257-1273.
- Christiansen JJ, Gravholt CH, Fisker S, et al. Very short term dehydroepiandrosterone treatment in female adrenal failure: impact on carbohydrate, lipid and protein metabolism. *Eur J Endocrinol.* 2005;152(1):77-85.
- Nair KS, Rizza RA, O'Brien P, et al. DHEA in elderly women and DHEA or testosterone in elderly men. *N Engl J Med.* 2006;355(16):1647-1659.
- Grimby G. Muscle performance and structure in the elderly as studied cross-sectionally and longitudinally. *J Gerontol A Biol Sci Med Sci.* 1995;50(special issue):17-22.
- Nair KS. Aging muscle. *Am J Clin Nutr.* 2005;81(5):953-963.
- Balagopal P, Schimke JC, Ades P, Adey D, Nair KS. Age effect on transcript levels and synthesis rate of muscle MHC and response to resistance exercise. *Am J Physiol Endocrinol Metab.* 2001;280(2):E203-E208.
- Short KR, Vittone JL, Bigelow ML, Proctor DN, Nair KS. Age and aerobic exercise training effects on whole body and muscle protein metabolism. *Am J Physiol Endocrinol Metab.* 2004 Jan;286(1):E92-E101. Epub 2003 Sep 23.
- Gurnell EM, Hunt PJ, Curran SE, et al. Long-term DHEA replacement in primary adrenal insufficiency: a randomized, controlled trial. *J Clin Endocrinol Metab.* 2008 Feb;93(2):400-409. Epub 2007 Nov 13.
- Villareal DT, Holloszy JO. DHEA enhances effects of weight training on muscle mass and strength in elderly women and men. *Am J Physiol Endocrinol Metab.* 2006 Nov;291(5):E1003-E1008. Epub 2006 Jun 20.
- Holloszy JO, Coyle EF. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J Appl Physiol.* 1984;56(4):831-838.
- Dhatariya K, Bigelow M, Nair KS. Effect of dehydroepiandrosterone replacement on insulin sensitivity and lipids in hypoadrenal women. *Diabetes.* 2005;54(3):765-769.
- Proctor DN, Beck KC. Delay time adjustments to minimize errors in breath-by-breath measurement of \dot{V}_{O_2} during exercise. *J Appl Physiol.* 1996;81(6):2495-2499.
- Stump CS, Short KR, Bigelow ML, Schimke JM, Nair KS. Effect of insulin on human skeletal muscle mitochondrial ATP production, protein synthesis, and mRNA transcripts. *Proc Natl Acad Sci U S A.* 2003 Jun 24;100(13):7996-8001. Epub 2003 Jun 13.
- DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol.* 1979;237(3):E214-E223.
- Nygren J, Nair KS. Differential regulation of protein dynamics in splanchnic and skeletal muscle beds by insulin and amino acids in healthy human subjects. *Diabetes.* 2003;52(6):1377-1385.
- Edwards RH. Percutaneous needle-biopsy of skeletal muscle in diagnosis and research. *Lancet.* 1971;2(7724):593-595.
- Nair KS, Welle SL, Halliday D, Campbell RG. Effect of β -hydroxybutyrate on whole-body leucine kinetics and fractional mixed skeletal muscle protein synthesis in humans. *J Clin Invest.* 1988;82(1):198-205.
- Nair KS, Ford GC, Ekberg K, Fernqvist-Forbes E, Wahren J. Protein dynamics in whole body and in splanchnic and leg tissues in type I diabetic patients. *J Clin Invest.* 1995;95(6):2926-2937.
- Perreault M, Dombrowski L, Marette A. Mechanism of impaired nitric oxide synthase activity in skeletal muscle of streptozotocin-induced diabetic rats. *Diabetologia.* 2000;43(4):427-437.
- Nair KS, Halliday D, Griggs RC. Leucine incorporation into mixed skeletal muscle protein in humans. *Am J Physiol.* 1988;254(2, pt 1):E208-E213.

26. Rooyackers OE, Adey DB, Ades PA, Nair KS. Effect of age on in vivo rates of mitochondrial protein synthesis in human skeletal muscle. *Proc Natl Acad Sci U S A*. 1996;93(26):15364-15369.
27. Rooyackers OE, Balagopal P, Nair KS. Measurement of synthesis rates of specific muscle proteins using needle biopsy samples. *Muscle Nerve Suppl*. 1997;5:S93-S96.
28. Balagopal P, Ljungqvist O, Nair KS. Skeletal muscle myosin heavy-chain synthesis rate in healthy humans. *Am J Physiol*. 1997;272(1, pt 1):E45-E50.
29. Ljungqvist OH, Persson M, Ford GC, Nair KS. Functional heterogeneity of leucine pools in human skeletal muscle. *Am J Physiol*. 1997;273(3, pt 1):E564-E570.
30. Nair KS. Assessment of protein metabolism in diabetes. In: Draznin B, Rizza R, eds. *Clinical Research in Diabetes and Obesity, Part 1: Methods, Assessment, and Metabolic Regulation*. Totowa, NJ: Humana Press; 1997:137-170.
31. Jones BN, Gilligan JP. o-Phthaldialdehyde precolumn derivatization and reversed-phase high-performance liquid chromatography of polypeptide hydrolysates and physiological fluids. *J Chromatogr*. 1983;266:471-482.
32. Short KR, Bigelow ML, Nair KS. Age effect on muscle mitochondrial function and impaired glucose tolerance after a mixed meal [abstract]. *Diabetes*. 2003;52(suppl 1):A346.
33. Short KR, Vittone JL, Bigelow ML, et al. Impact of aerobic exercise training on age-related changes in insulin sensitivity and muscle oxidative capacity. *Diabetes*. 2003;52(8):1888-1896.
34. Arlt W, Callies F, van Vlijmen JC, et al. Dehydroepiandrosterone replacement in women with adrenal insufficiency. *N Engl J Med*. 1999;341(14):1013-1020.
35. Long W, Barrett EJ, Wei L, Liu Z. Adrenalectomy enhances the insulin sensitivity of muscle protein synthesis. *Am J Physiol Endocrinol Metab*. 2003 Jan;284(1):E102-E109. Epub 2002 Oct 1.
36. Wallace MB, Lim J, Cutler A, Bucci L. Effects of dehydroepiandrosterone vs androstenedione supplementation in men. *Med Sci Sports Exerc*. 1999;31(12):1788-1792.
37. Igwebuie A, Irving BA, Bigelow ML, Short KR, McConnell JP, Nair KS. Lack of dehydroepiandrosterone effect on a combined endurance and resistance exercise program in postmenopausal women. *J Clin Endocrinol Metab*. 2008 Feb;93(2):534-538. Epub 2007 Nov 20.
38. Brodsky IG, Balagopal P, Nair KS. Effects of testosterone replacement on muscle mass and muscle protein synthesis in hypogonadal men—a clinical research center study. *J Clin Endocrinol Metab*. 1996;81(10):3469-3475.
39. Bhasin S, Storer TW, Berman N, et al. The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. *N Engl J Med*. 1996;335(1):1-7.
40. Forbes GB. The effect of anabolic steroids on lean body mass: the dose response curve. *Metabolism*. 1985;34(6):571-573.
41. Welle S, Bhatt K, Thornton CA. High-abundance mRNAs in human muscle: comparison between young and old. *J Appl Physiol*. 2000;89(1):297-304.
42. Balagopal P, Rooyackers OE, Adey DB, Ades PA, Nair KS. Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcomeric protein in humans. *Am J Physiol*. 1997;273(4, pt 1):E790-E800.
43. Short KR, Vittone JL, Bigelow ML, et al. Changes in myosin heavy chain mRNA and protein expression in human skeletal muscle with age and endurance exercise training. *J Appl Physiol*. 2005 Jul;99(1):95-102. Epub 2005 Mar 3.
44. Tagliaferro AR, Ronan AM, Payne J, Meeker LD, Tse S. Increased lipolysis to β -adrenergic stimulation after dehydroepiandrosterone treatment in rats. *Am J Physiol*. 1995;268(6, pt 2):R1374-R1380.
45. Dairaghi DJ, Shadel GS, Clayton DA. Human mitochondrial transcription factor A and promoter spacing integrity are required for transcription initiation. *Biochim Biophys Acta*. 1995;1271(1):127-134.
46. Gordon SE, Flück M, Booth FW. Plasticity in skeletal, cardiac, and smooth muscle: selected contribution: skeletal muscle focal adhesion kinase, paxillin, and serum response factor are loading dependent. *J Appl Physiol*. 2001;90(3):1174-1183.
47. Woelfle J, Chia DJ, Massart-Schlesinger MB, Moyano P, Rotwein P. Molecular physiology, pathology, and regulation of the growth hormone/insulin-like growth factor-I system. *Pediatr Nephrol*. 2005 Mar;20(3):295-302. Epub 2004 Nov 10.