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DHEA replacement in hypoadrenal women - investigation on protein anabolism and skeletal muscle function

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Abstract

Objective—To determine whether dehydroepiandrosterone replacement in hypoadrenal women improves performance, muscle protein accretion and mitochondrial functions.

Participants and methods—Thirty-three hypoadrenal women were enrolled between 1st May 2002 and 31st May 2003. 28 completed a 12 week prospective randomized, placebo-controlled, crossover study with either daily placebo or 50 mg DHEA with a 2-week of washout, and then crossed over to the other treatment. Body composition, physical performance, whole body and muscle protein metabolism and mitochondrial functions were determined.

Results—DHEA administration significantly increased plasma levels of DHEA-S, 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 mRNA levels of genes encoding mitochondrial proteins and nuclear transcription factors did not change following DHEA administration. However, mRNA levels of muscle myosin heavy chain isoform 1 (MHC I) ($P<0.005$) which determines muscle fiber type as well as those of IGF Binding Proteins 4 and 5 significantly decreased ($P<0.04$ respectively).

Conclusion—Three months of DHEA increased DHEA-S and androgen levels but had no impact on physical performance, body composition, protein metabolism or on muscle mitochondrial biogenesis in hypoadrenal women. However, lowering of mRNA levels of binding proteins of IGF 1 and MHC I suggest potential effects of DHEA on muscle fibre type on long term treatment.

Key terms

Dehydroepiandrosterone; hypoadrenal; muscle strength

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INTRODUCTION

The 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 decline by 10% per decade thereafter¹⁻⁴. As a result healthy elderly men and women have lower DHEA and DHEA-S levels than younger, healthy individuals. Levels are very low or almost undetectable in women with primary hypoadrenalism due to Addison's disease, or in those who have had 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 tissues⁵. Its other physiologic functions in healthy adults remain unclear. A recent review looking at the epidemiological associations and effects of replacement of DHEA on body composition, bone density, and muscle function has shown conflicting reports⁶.

Several studies have been carried out looking at the effect of DHEA replacement on skeletal muscle strength and lean body mass in individuals with low DHEA levels, either the well elderly, or those who are hypoadrenal^{7,8}. The study in elderly people with low DHEA-S demonstrated no improvement of physical performance, body composition, insulin sensitivity or bone density⁸. However, the studies done in the elderly can not be translated to the hypoadrenal people because unlike in the elderly the hypoadrenal people have very low or undetectable DHEA-S levels. With aging parallel to the decline in DHEA-S levels there is a reduction in skeletal muscle oxidative capacity, muscle protein synthesis and a decline in exercise performance^{9,10}. It remains to be determined whether DHEA replacement improves these muscle functions in hypoadrenal women with very low DHEA-S.

The very low or absent levels of DHEA or DHEA-S found in women who have Addison's disease or who have been adrenalectomized may contribute to their decline in muscle mass and function. Although short term resistance exercise programs significantly improve skeletal muscle mass¹¹, it is possible that short term therapy with DHEA may not show small changes in muscle mass, and are thus not detected in many previous studies. However, measurement of muscle protein synthesis responds more acutely to short term intervention and may be a better method of detecting these changes¹². Recent work looking at DHEA replacement in an Addisonian population showed an increase in truncal lean body mass¹³, with other authors showing that DHEA given to a well elderly population led to increasing strength after weight training¹⁴ however, no detailed explanation for possible mechanisms were offered. The present study measured muscle protein synthesis rates to determine whether DHEA replacement exerted its effects on skeletal muscle protein synthesis rates, accounting for this anabolic effect. Muscle mitochondrial function decline in parallel to DHEA levels in elderly people. However, the aerobic exercise programs of relatively short term period improved mitochondrial oxidative capacity in the elderly people¹⁵. In the current study we assessed whether three months of DHEA administration in subjects who were absolutely DHEA deficient increases muscle improves their mitochondrial biogenesis.

Muscle functions and performance depend on mitochondrial function and the composition of contractile proteins such as the isoforms of myosin heavy chain. We therefore determined whether DHEA therapy improves muscle mitochondrial enzymes, mRNA levels of isoforms of myosin heavy chain, mitochondrial-related proteins, transcription factors, and fractional synthesis rates of mitochondrial and sarcoplasmic fraction of muscle proteins.

The purpose of the present investigation therefore, was to test the hypothesis that 12 weeks of DHEA replacement in hypoadrenal women with profoundly low levels of DHEA would improve muscle strength, and muscle metabolic processes. In addition, these women were placed on a standardized glucocorticoid replacement regimen.

METHODS

Ethical information

The study protocol and consent form were approved by Mayo Clinic Institutional Review Board (IRB). All experiments conducted are conformed to the standards set by the latest revision of Declaration of Helsinki. All volunteers signed informed IRB approval consent forms.

Subjects

Participant characteristics, inclusion and exclusion criteria have been previously described in a paper that demonstrated that DHEA increased insulin sensitivity¹⁷. Thirty-three hypoadrenal women were randomized and 28 completed the entire study. The five remaining subjects withdrew for a variety of reasons - 3 subjects withdrew without completing the first arm of the study (1 subject withdrew due to diarrhoea (on DHEA), 1 subject unblinded herself (on DHEA), 1 subject withdrew citing pressure of work (on DHEA). 2 subjects withdrew after completing the first arm of the study (both on placebo).

Of the 28 subjects who completed the study, the mean ($\pm$ SD) age was 50.25 ± 15.9 years and their BMI ($\pm$ SD) was 27.0 ± 4.4 Kg/m². 14 subjects were post menopausal, with the mean length of time being hypoadrenal 12.25 ± 1.9 years. 20 subjects (61%) had primary Addison's disease, with other diagnoses including bilateral adrenalectomy due to Cushing's syndrome (n = 5), benign bilateral pheochromocytomas (n = 1), and congenital adrenal hyperplasia (n = 1).

Study Protocol

This was a single center, randomized, placebo-controlled, crossover design. The same subjects received twelve weeks each of both placebo and DHEA. 50 mg of pharmaceutical grade micronized DHEA (Gardena, CA) 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 haematology and biochemistry tests were conducted to assess general health. Further blood tests included DHEA-S, androstenedione, and bioavailable testosterone. Prior to the first phase of the study subjects underwent a standard submaximal treadmill exercise stress test to exclude cardiorespiratory abnormalities.

All subjects received hydrocortisone given in divided doses (mean total daily dose 24.9 ± 7.1 mg) in order to minimize the potentially confounding effects of different glucocorticoids replacement regimens. Eight subjects had previously been on prednisone (mean daily dose 4.5 ± 1.4 mg).

At the end of the first period on the drug, the subjects were admitted to the General Clinical Research Center (GCRC or the current name Clinical Research Unit of Mayo Clinic Clinical and Translational Activity Center or CTSA) at Mayo Clinic.

Subjects had indirect calorimetry measured for 30 minutes. Subjects then underwent VO₂ max testing by the use of a stationary bicycle¹⁸.

Subjects performed upper and lower body strength tests. A one-repetition maximum (1RM) was established for seated chest press, bicep curl, knee extension, and leg curl. Subjects performed a series of 1 to 3 repetitions, at higher resistance loads, until the 1RM was established. 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 12 weeks of the alternate preparation. Subjects 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 volunteers¹⁹. For this reason, as well as to assess insulin sensitivity,¹⁷ a hyperinsulinemic (1.5 mU/kg FFM /min) euglycemic clamp was used in the present study^{20;21}.

Of the 28 subjects, 7 were studied with additional infusions of [¹⁵N] phenylalanine and [ring 2, 3,5,6-²H₄]tyrosine (²H₄ tyrosine) to measure amino acid kinetics and fractional synthesis rates of muscle mitochondrial proteins and sarcoplasmic protein subfractions. In the subjects in whom amino acids were infused, priming doses of [¹⁵N] phenylalanine at 1.6 mg/Kg FFM, [¹⁵N] tyrosine at 0.6 mg/Kg FFM and [²H₄] tyrosine at 0.6 mg/Kg FFM were given at 04:00. Infusions of ¹⁵N phenylalanine and [²H₄] tyrosine were then continued at 1.6 mg/Kg FFM/hr and 0.6 mg/Kg FFM/hr respectively. Infusions lasted for 10 hours, with skeletal muscle needle biopsies being taken at 3 hours and 10 hours after the start of the tracer infusions.

Muscle Biopsies

Vastus lateralis muscle samples (~300 mg each) were obtained under local anaesthesia, with a percutaneous needle as previously described^{22;23}. The first biopsy was done prior to the start of the insulin infusion at 07:00. The second biopsy was taken from the contralateral thigh after 7 hours.

Whole Body Protein Turnover and Muscle Protein Synthesis—Plasma isotopic enrichment [¹⁵N]phenylalanine, [¹⁵N]tyrosine, and [²H₄] tyrosine were measured using a gas chromatograph/mass spectrometer (as previously described)^{24;25}. Calculation of phenylalanine flux, tyrosine flux, phenylalanine conversion to tyrosine and phenylalanine incorporation into protein (protein synthesis) were also performed as previously described^{24;25}. A 150 mg portion of each muscle sample was used for the isolation of mitochondrial and sarcoplasmic protein fractions by differential centrifugation as previously described^{23;26–28}. Isotopic enrichments of [¹⁵N]Phe in muscle proteins were determined using a gas chromatograph (GC)-combustion-isotope ratio mass spectrometer (MS) (Delta Plus, Finigan MAT) as previously described²⁹. Muscle tissue fluid tracer enrichment and plasma amino acid enrichment were measured as previously described³⁰.

The fractional synthesis rate (FSR) of mixed muscle protein, mitochondrial and sarcoplasmic proteins was calculated using the equation³¹: $FSR (\%/h) = 100 \times (E_{10h} - E_{3h}) / (E_{TF} \times t)$ 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 biopsies, and t is the time of incorporation between the two biopsies – in this study 7 hours (mean 6.9 hours, 95% CI 6.82, 7.04).

Mitochondrial Enzyme Activities

Maximal enzyme activities of citrate synthase and cytochrome c oxidase were measured in the tissue homogenate and in the mitochondrial pellet by use of photospectrometric analyses as previously described^{19;27}. 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 by a high-performance liquid chromatography system (HP 1090, 1046 fluorescence detector and cooling system)³². Glucose was analyzed on site by using an enzymatic technique (Beckman Instruments Inc., Fullerton, CA).

Hormonal Assays

Free and total IGF-1, IGFBP-1, IGFBP-3 and SHBG were measured using a two-site immunoradiometric assay. DHEA-S was measured using a competitive RIA, with androstenedione measured by direct RIA. These assays were from Diagnostic Systems Laboratories (Webster, TX). Testosterone was measured with the coat-a-count RIA (DPC, Los Angeles, CA).

mRNA Quantification

RNA extractions were performed on frozen muscle samples from individual subjects as previously described^{11;16;19}. A real-time quantitative polymerase chain reaction (PCR) system (PE Biosystems, Foster City, CA) was used to measure Myosin Heavy Chain (MHC) I, MHC II, and mitochondrial transcription factor A (TFAM), peroxisome proliferators – activative receptor – γ co-activator 1–2 (PGC1 α), cytochrome c oxidase (COX) subunits 3 and 4, and NADH subunit 4 mRNAs. The signal for the 28S rRNA 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 curve¹¹. The sequences for MHC oligos have been previously published¹¹, as has TFAM^{16;33}.

Statistical Analysis—Randomization of treatment orders assigned to subjects was done independently by the division of biostatistics at Mayo Clinic. Because this was a crossover study, subjects acted as their own controls.

The data presented in this paper was part of a larger study that also looked at the effects of DHEA replacement on the mood, memory and sexual wellbeing of hypoadrenal women. The power calculation for the larger overall study was based on previous data using the visual analogue scale for sexuality assessment used by Arlt et al [personal communication]. They showed changes in components of the scales of sexual activity had standard deviations ranging from 23.8 to 29.9. Based on this, paired t-tests at the 5% level have 80% power to detect changes of 13.9 to 19.02, or 40.3 to 50.7% of baseline values with 25 subjects. Arlt et al observed within subject changes of this magnitude in subjects on DHEA treatment³⁴.

Analyses were done to assess changes of endpoints comparing changes in skeletal muscle physiology after 12 weeks on placebo and 12 weeks on DHEA. Paired t test and Wilcoxon signed rank sum test were used for analyses. Data is given as mean $\pm$ standard deviations.

Pearson product correlations were used to determine the strength of association for selected variables. Significant effect for all tests was accepted at $P < 0.05$.

Results

Hormone Levels

The hormonal results have previously been published in Table 1 in reference¹⁷. In summary, DHEA treatment significantly increased serum DHEA-S levels. There were also significant increases in bioavailable testosterone and androstenedione, whereas the level of SHBG was reduced by DHEA treatment.

Body composition, Strength tests, Exercise Performance and Indirect calorimetry tests

DHEA treatment had no effect on percentage fat, fat free mass, bone mineral density, hand grip, biceps curl, chest press, leg curl and leg press representing upper and lower extremity strength (Table 1). Indirect calorimetry, respiratory quotient and resting energy expenditure showed no changes in response to DHEA treatment. Stationary bike testing of maximal oxygen consumption, peak bike power, and heart rate also showed no significant differences between DHEA and placebo. Data are shown in Table 2.

Protein Turnover, Protein Fractional Synthesis Rates 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 proteins, representing protein synthesis (Table 3). Fractional synthesis rates of sarcoplasmic proteins and mitochondrial proteins are shown in Table 3 showing no differences between the two studies. Muscle mRNA levels of mRNA for TFAM and for myosin isoforms were measured by real-time quantitative PCR. In contrast mRNA levels of PGC1 α , TFAM, NRF-1, COX3, COX4, and NADH4 did not change with treatment (data not shown). We also found no differences in cytochrome c oxidase (89.22 ± 22.48 μ U/g protein, placebo vs 96.35 ± 13.11 DHEA, $p=0.55$) and citrate synthase activity (137.07 μ U/g protein, placebo vs 142.11 ± 35.66 , DHEA, $p=0.46$) in muscle. There was a significant decrease in MHC I mRNA in response to DHEA treatment (Figure 1) but no significant differences were noted for MHC IIa and x. A significant decline in mRNA levels IGFBP₄ and BP₅ were noted but no changes in IGF1 or androgen receptor.

DISCUSSION

The current study has shown that 50 mg of once daily DHEA administration in hypoadrenal women aged 20 to 80 years for three months had no significant effects on energy metabolism, peak VO₂, muscle strength, muscle protein synthesis, or mitochondrial enzymes. However, DHEA replacement reduced skeletal muscle mRNA expression of myosin heavy chain isoform 1 (MHC-1), IGFBP₄ and BP₅ but had no effect on mRNA levels of selected nuclear and mitochondrial genes encoding mitochondrial proteins and transcription factors. The increase in DHEA-S levels on DHEA replacement in these women¹⁷ were similar to the high levels that are commonly observed in young people⁸.

Very little work has been done on physical performance or skeletal muscle protein synthesis rates in hypoadrenal women. Animal work has shown that adrenalectomy *per se* does not affect skeletal muscle protein synthesis rates³⁵. The lack of effect of DHEA on muscle function in our study is in agreement with previous studies done in elderly subjects with low DHEA but treated with DHEA^{8;36}. However, it contradicts findings from a recent study that showed that supplemental DHEA for six months in the elderly modestly enhances resistant exercise-induced increases in muscle mass¹⁴ but is consistent with another study in post-menopausal women in whom DHEA administration offered no additional benefits to the exercise program³⁷.

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 men³⁸ but the current study showed that despite an increase in androgens including bioavailable testosterone levels DHEA replacement had no effect on muscle protein synthesis in hypoadrenal women. There are two potential explanations on why modest increase in testosterone did not stimulate muscle protein synthesis. Firstly, it is possible that testosterone effect on muscle protein synthesis is dose dependent and that higher testosterone levels may

have increased muscle protein synthesis. A dose effect of testosterone has been previously reported^{39;40}. Secondly, it is possible that testosterone effect on muscle protein synthesis does not occur in women.

The decline in MHC I mRNA with DHEA replacement is of great interest given that aging (a 'DHEA deficiency state') is associated with a decrease in MHC IIa and MHC IIx mRNA expression with no effect on MHC I^{41;42}. In type 1 muscle fibers MHC I protein expression is higher and the relative abundance of MHC I protein and mRNA levels are higher in older people^{11;43}. The functional implication of the current observation showing a decline in MHC I mRNA expression in muscle on DHEA replacement is not forthcoming from the current study. However, it is possible that being a protein with a slow synthesis rate,⁴² alteration of composition of MHC I 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}. Lack of any effect of DHEA on energy metabolism at whole body level is consistent with the observation in muscle mitochondrial metabolism. Muscle mitochondrial function as assessed by enzyme activities were not affected by DHEA replacement. The enzymes cytochrome c oxidase and citrate synthase were measured because they form an integral part of the electron transport chain and ATP production. Mitochondrial enzyme activity can be used as a surrogate for ATP production capacity²⁷, thus it is unlikely that DHEA affects ATP production. This may account for the lack of change in physical performance seen with peak VO₂ or skeletal muscle strength. Moreover, mRNA levels of mitochondrial proteins encoded by nuclear and mitochondrial genes also were not altered by DHEA. The mRNA levels of mitochondrial transcription factors, PGC-1 α , NRF-1 and TFAM also remained unchanged between the DHEA and placebo arms of the study. All the above are an integral part of the mechanism responsible for mitochondrial DNA (mtDNA) transcription and replication⁴⁵. These transcription factors have been shown to be a key regulator of exercise induced mitochondrial gene expression, and thus oxidative capacity⁴⁶. The failure to find a change in mRNA levels of these nuclear transcription factors with DHEA replacement is consistent with the unchanged mitochondrial function that was observed.

Of note, while mRNA levels of IGF 1 did not change, decrease in mRNA levels of IGFBP 4 and 5 occurred. The expression of these proteins play key roles in the biological action of IGF 1 in muscle⁴⁷. Although plasma levels of either IGF 1 or its binding proteins (IGFBP 1 and BP3) did not change on DHEA administration¹⁷, the potential effect of IGF1 in muscle on long-term cannot be excluded.

There are some limitations to our study. All of our volunteers were Caucasian and most came from a tertiary referral center, and as such there is a possibility of referral bias. However, we were able to recruit over a third (n = 10) from individuals who had never been seen at Mayo Clinic. It is difficult to know if there were systematic differences between non-responders to our invitations to participate in the study and those who took part. This study does include a wide range of ages and the results should therefore be applicable to Caucasian hypoadrenal women over 18 years old, and are likely to be representative of the female hypoadrenal population as a whole. Whether these results can be applied to other groups, such as males, or those with low DHEA levels with normally functioning adrenal glands, remains open to question. The importance of the current study is that 3 months of maintenance of DHEA-S levels in DHEA deficient women offered an opportunity to assess the physiological role of DHEA in women, who, unlike men, have very lower androgen levels.

In summary, the current study has demonstrated that 50 mg of DHEA given once daily for three months to hypoadrenal Caucasian women on standardized glucocorticoid replacement 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 and muscle mitochondrial biogenesis were noted on DHEA replacement. However, DHEA administration reduced the mRNA expression of IGF binding proteins 4 and 5 as well as MHC I indicating potential long-term physiological and anatomical effect.

DHEA remains freely available over the counter in the USA as a 'health food supplement' unlike in Europe. This is despite the fact that DHEA is not a food, does not naturally appear in the human food chain, and no foodstuff can carry out the physiological role of DHEA. Although the previous finding showed that DHEA modestly increases insulin sensitivity and favorably alters lipid profile in these individuals¹⁷, the results of the present study does not show any appreciable effects of DHEA on physical performance in hypoadrenal women. The current study however, was of three months duration and the long term effects cannot be fully determined, since the results from the current study indicates potential effects on muscle fiber type and IGF-1 action.

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Abbreviations used

1RM, 1 Repetition Maximum
 BMI, Body Mass Index
 COX, cytochrome c oxidase
 DHEA, Dehydroepiandrosterone
 DHEA-S, Dehydroepiandrosterone Sulfate
 E_{10h}, Amino acid enrichment in muscle protein at 10 hours
 E_{3h}, Amino acid enrichment in muscle protein at 3 hours
 E_{TF}, Average enrichment of amino acids in muscle tissue fluid taken from the 3 and 10-hour biopsies
 FFM, Fat Free Mass
 FSR, Fractional Synthesis Rate
 IGF, Insulin Like Growth Factor
 IGFBP, Insulin Like Growth Factor Binding Protein
 MHC, Myosin Heavy Chain
 mRNA, Messenger Ribonucleic Acid
 NRF-1, nuclear respiratory factor 1
 PCG-1 α , peroxisome proliferators-activated receptor (PPAR)- γ coactivator 1- α
 PCR, Real-time quantitative polymerase chain reaction
 SHBG, Sex Hormone Binding Globulin
 TF, Tissue Fluid
 TFAM, mitochondrial transcription factor A
 VCO₂, CO₂ consumption

VO₂, O₂ consumption

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Figure 1.

Skeletal muscle mRNA levels showing that DHEA administration significantly decreased IGFBP₄, IGFBP₅ and MHC1 mRNA levels with no significant effects on those of IGF-1, MHCIIa and IIx. * = indicates statistical significance DHEA = Dehydroepiandrosterone, IGF = Insulin Like Growth Factor, IGFBP = Insulin Like Growth Factor Binding Protein, MHC = Myosin Heavy Chain, mRNA = Messenger Ribonucleic Acid

Effect of dehydroepiandrosterone Replacement on Body Composition, and Muscle Strength. Unless otherwise stated n = 28. One subject was unable to lie down for the biceps curl and was thus not included in that analysis. During the assessment visit for one of the subjects, the leg curl machine was out of order, thus her data was not included in the analysis.

TABLE 1

BODY COMPOSITION AND BONE	Placebo			DHEA			P value (cross over design)	P value (paired t test)	80% power to detect difference*
	n	mean	SD	n	mean	SD			
Fat (%)	27	45.4	5.76	28	44.78	6.46	0.25	0.24	1.68
Fat Free mass (kg)	28	39.7	4.69	28	39.81	4.56	0.6	0.61	0.78
Bone Density (g/cm ²)	28	1.2	0.1	28	1.17	0.1	0.69	0.63	0.01
Hand Grip Strength (kg)	27	29.3	5.85	28	29.05	5.92	0.7	0.75	1.58
Bicep Curl (kg)	27	37.5	6.89	27	37.71	7.7	0.89	0.69	1.59
Leg Press (kg)	28	57.3	15.85	28	57.35	14.8	0.98	0.97	3.19
Chest Press (kg)	27	40.0	8.59	27	40.49	9.14	0.59	0.47	1.81
Leg Curl (kg)	27	39.6	11.26	27	39.81	11.28	0.63	0.7	1.86

TABLE 2

Indirect calorimetry data (VCO_2 and CO_2 production and VO_2 , O_2 consumption) aerobic performance (peak VO_2 , peak bike power and peak heart rate). $\text{VCO}_2 = \text{CO}_2$ consumption, $\text{VO}_2 = \text{O}_2$ consumption

Variable	Placebo			DHEA			P value (cross over design)	P value (paired t test)	80% power to detect difference
	n	mean	SD	n	mean	SD			
Indirect Calorimetry resting VCO_2 (ml/min)	28	170.2	20.2	28	170.5	20.45	0.97	0.94	11.05
Indirect Calorimetry resting VO_2 (ml/min)	28	205.1	27.04	28	204.96	24.92	0.96	0.98	10.06
Respiratory Quotient	28	0.8	0.06	28	0.83	0.06	0.95	0.95	0.04
Resting Energy Expenditure (kcal/day)	28	1421.5	176.4	28	1418.3	167.17	0.85	0.9	68.01
Peak Bike Power (Watts)	27	119.8	35.67	28	115.89	34.37	0.15	0.16	5.91
Peak VO_2 per Kg FFM (ml/min/kg FFM)	28	36.7	8.44	28	36.34	7.42	0.67	0.62	2.03
Peak Heart Rate (beats per minute)	26	160.0	21.45	28	161.32	21.66	0.13	0.13	7.25

TABLE 3

Effect of dehydroepiandrosterone replacement on whole body phenylalanine and tyrosine flux values and FSR or mitochondrial sarcoplasmic and mixed muscle proteins. These FSR calculations were performed using muscle tissue fluid ($^{13}\text{C}_6$) phenylalanine enrichment as precursor pool ³⁰.

	Placebo Mean ($\pm$ SD)	DHEA Mean ($\pm$ SD)	p value paired t test	p value crossover design	80% power to detect difference
Phenylalanine flux ($\mu\text{mol/kg/hr}$)	55.4 (5.03)	52.2 (5.8)	0.07	0.43	5.81
Tyrosine flux ($\mu\text{mol/kg/hr}$)	45.4 (4.4)	44.6 (4.2)	0.68	0.49	5.94
Phenylalanine conversion to tyrosine ($\mu\text{mol/kg/hr}$)	5.0 (1.03)	4.6 (0.7)	0.52	0.76	2.18
Phenylalanine incorporation to protein ($\mu\text{mol/kg/hr}$) (protein synthesis)	50.3 (4.8)	47.6 (5.5)	0.07	0.33	5.34
Mitochondrial FSR (%/hr)	0.187 (0.108)	0.158 (0.042)	0.64	0.75	0.03
Sarcoplasmic FSR (%/hr)	0.118 (0.061)	0.112 (0.027)	0.77	0.71	0.02
Mixed Muscle Protein FSR (%/hr)	0.057 (0.02)	0.053 (0.01)	0.62	0.75	0.03

* Denotes a percentage change in level. FSR-fractional synthesis rate, TF-tissue fluid.