Assessing the Effects of Dehydroepiandrosterone (DHEA) Replacement on Insulin Sensitivity, Body Composition, and Skeletal Muscle Physiology in Hypoadrenal Women

A Thesis Submitted to the University of London

By

Dr Ketan Kumar Dhatariya

In Partial Fulfilment of the Requirements for the MD

February 2004

Abstract

Background:

Dehydroepiandrosterone (DHEA) and its sulphated ester (DHEAS) are the most abundant steroid hormones found in the circulation. The physiological function of these hormones has yet to be determined.

DHEA and DHEAS are produced by the adrenal gland. DHEA replacement is not part of the standard of medical care for hypoadrenal subjects.

Previous work has shown that DHEA can lead to changes in body composition, insulin sensitivity, and skeletal muscle strength.

Methods:

In a single centre, randomised, double blind, placebo controlled, cross over study 33 hypoadrenal women (± SD) (mean age 47.9 ± 15.6 years) were given 50 mg of DHEA as a single daily dose, and identically encapsulated placebo, for 3 months each. Subjects had assessments of body composition by DEXA, physical function by bicycle VO_2 max testing, and skeletal muscle strength testing. Insulin sensitivity was assessed by hyperinsulinaemic euglycaemic clamping. Skeletal muscle needle biopsies were taken to assess the effects of DHEA replacement on mitochondrial enzyme activity, and synthesis rates of mixed muscle protein, mitochondrial, and sarcoplasmic proteins.

Results:

There were no differences in body composition as assessed by measurements of fat mass, fat free mass and bone density. Insulin sensitivity was significantly improved with DHEA compared with placebo. The M value was higher with DHEA supplementation (1.34 ± 0.53 vs 1.22 ± 0.52 mg/min/KgFFM, p = 0.02) despite comparable glycaemic control. Fasting plasma insulin and glucagon levels were significantly lower after 12 weeks of DHEA supplementation (7.07 ± 4.35 vs 8.88 ± 5.81 μU/ml, p = 0.003 and 51.06 ± 17.2 vs 56.03 ± 22.85 pmol/l, p = 0.038 respectively). There was a strong trend to lowering fasting glucose (4.67 ± 0.54 vs 4.83 ± 0.58 mmol/l, p = 0.054).

This study also demonstrated significant reductions in total cholesterol, (4.62 ± 0.9 vs 5.23 ± 0.83 mmol/l, p = 0.007), triglycerides, (1.52 ± 0.7 vs 1.70 ± 0.80 mmol/l, p = 0.016), and HDL cholesterol, (0.97 ± 0.32 vs 1.09 ± 0.33 mmol/l, p = 0.003) with DHEA vs placebo.

The present study showed no change in mitochondrial enzyme activity (cytochrome c oxidase 96.35 ± 31.11 vs 89.22 ± 22.48 uU/g protein, p = 0.547, and citrate synthase 142.11 ± 35.66 vs 137.07 ± 36.63 uU/g protein, p = 0.461, DHEA vs placebo respectively). There were no changes in skeletal muscle protein synthesis rates with DHEA vs placebo, (mixed muscle protein 0.0529 ± 0.009 vs 0.057 ± 0.02 %/hr, p = 0.375, mitochondrial protein 0.053 ± 0.087 vs 0.0569 ± 0.02 %/hr, p = 0.375, sarcoplasmic protein 0.0378 ± 0.006 vs 0.0363 ± 0.009 %/hr, p = 0.813, DHEA vs placebo respectively).

In addition, there were no changes in any measures of physical capacity, measured by strength testing or by bicycle VO_2 max assessments.

Conclusion:

This is the first study to show that DHEA replacement significantly increases insulin sensitivity in hypoadrenal subjects. These results may be significant in hypoadrenal populations where insulin treatment has been shown to improve morbidity and mortality, e.g. intensive care.

This study also demonstrated significant reductions in total cholesterol, HDL, and triglycerides. These reductions may be part of the reason for the observations seen in epidemiological studies correlating reduced cardiovascular mortality with high DHEA levels.

This study also showed no demonstrable effect on skeletal muscle fractional synthesis rates or mitochondrial enzyme activity, thus suggesting possible explanations for the lack of effect of DHEA replacement on body composition, or skeletal muscle performance during exercise.

Table of Contents

Table of Figures

List of Tables

Acknowledgements

This work was made possible by support from the National Institutes of Health. In particular, GCRC grant M01-RR00585 and Dr Nair's individual grant, AG14383-05.

I am very grateful to my mentors, Dr Sree Nair, Dole Professor of Nutrition and Consultant in Endocrinology in the Endocrine Research Unit at Mayo Clinic, Rochester, Minnesota, and Dr Margot Umpleby, Reader in Human Metabolism in the department of Diabetes, Endocrinology and Internal Medicine at St Thomas' Hospital, London. They have steered me through the difficult transformation from full time clinician to researcher and back again.

I would also like to thank Maureen Bigelow, RN and Dr Kevin Short, PhD for their invaluable help and guidance for the practical aspects of this study.

My thanks go to the skilled technical staff of Dr Nair's laboratory, Jane Kahl, Dawn Morse, Jill Schimke, Kate Windebank, and Paul Rys. I would also like to acknowledge the assistance of Jean Feehan, RN and Barb Norby, RN for their help with the inpatient stays.

I am grateful for the help, ideas and assistance given to me by the other fellows in Dr Nair's laboratory, Dr Dan Short MD PhD, Dr Ada Igwebuike MD, Dr Laura Greenlund MD PhD, Dr Sreekumar Raghavkaimal PhD, Dr Jothydev Kesavadev MBBS, Dr Yan Asmann PhD, and Dr Abdul Jaleel PhD.

My gratitude goes to Dr Charles Ford PhD, Mrs Mai Persson and Larry Ward who were responsible for the processing of samples for mass spectroscoptic analysis. They were responsible for providing me with the data once I gave them the samples.

Dr Ann Oberg PhD, was invaluable in the statistical analysis of the study, both in setting up the study, and in advising me on analysing the results.

I am also indebted to the staff of the General Clinical Research Center at St Marys Hospital, Rochester, Minnesota, for their help, patience, and understanding.

My thanks go also to all of the people who helped me to recruit, in particular Joan Hoffman at Addison News, and Erin Foley at the National Adrenal Diseases Foundation. I would especially like to thank all of the volunteers in the study, including those who failed the screens. Without their belief that progress is only made through research, none of this would have been possible.

My deepest thanks to Hilary Lane in the history of medicine library at Mayo Clinic, Rochester, for allowing me ungloved access to one of only 6 existing copies of the 1543 edition of De Humani Corporis Fabrica by Vesalius. A truly unique experience, which I shall not forget in many, many years. Figure 1 is scanned from that volume, courtesy of the department of medical illustration.

I am particularly grateful to my wife, Vasu, for having given up so much to allow me to pursue my selfish need for personal advancement, and for her unwavering faith in my ability, especially on those numerous occasions when I have doubted myself.

Lastly, I thank my beloved daughter, Ishika, for bringing me back down to earth every day by seeing me only as a mobile climbing frame and, unlike so many others, has no other aspirations of me whatsoever.

Rochester, September 2003

London, February 2004

Abbreviations Used in This Dissertation

ATP - Adenosine Triphosphate

BA - Bilateral Adrenalectomy

BMD - Bone Mineral Density

BMI - Body Mass Index

C - Cross-over Design

COX - Cytochrome c Oxidase

CS - Cushing's Syndrome

DEXA - Dual Energy X-Ray Absorptiometry

DHEA - Dehydroepiandrosterone

DHEAS - Dehydroepiandrosterone Sulphate

DHEA(S) - Both Dehydroepiandrosterone and Dehydroepiandrosterone Sulphate

ECG - Electrocardiogram

FFM - Fat Free Mass

FSR - Fractional Synthesis Rate

GCRC - General Clinical Research Center

GLUT - Glucose Uptake Transporter

HDL - High Density Lipoprotein

IGF - Insulin Like Growth Factor

IGF BP - Insulin Like Growth Factor Binding Protein

Kg - Kilogram

LDL - Low Density Lipoprotein

MHC - Myosin Heavy Chain

mRNA - Messenger Ribonucleic Acid

mtDNA - Mitochondrial DNA

N/A - Not Applicable

O - Observational

OL - Open Label

P - Placebo Controlled

R - Randomised Trial

RER - Respiratory Exchange Ratio

1 RM - One Repetition Maximum

RQ - Respiratory Quotient

VO_2 Max - Maximal Oxygen Consumption

VLDL - Very Low Density Lipoprotein

Background and Introduction

Dehydroepiandrosterone (DHEA) and its sulphated ester (DHEAS) [together known as DHEA(S)] have become increasingly fashionable over the past few years with more and more evidence emerging as to their possible roles in normal human physiology. Recent data has shown DHEA(S) levels to be highly correlated to longevity in non-human primates [1], and headline phrases such as 'mother of all hormones', 'superhormone', 'fountain of youth' and 'a therapy to restore body, pep of youth' [2] have brought these hormones to the attention of the lay public. Entering 'DHEA' into the internet search engine Google (www.google.com) in the last week of January 2004, revealed over 600,000 websites mentioning this hormone.

Low or absent levels of DHEA(S) are found in healthy elderly individuals and hypoadrenal subjects. Long term trials of DHEA replacement in these groups of people are currently in progress in the UK and in the USA, however these studies are not due to report for some time. With the availability of DHEA over the counter in the USA, and on the internet worldwide, a need was perceived for a properly conducted study to help clarify of some of the claims made about this hormone on muscle function in those people in whom DHEA levels are low.

The Early Anatomical History of the Adrenal Gland

The adrenal glands were initially unrecognised by early anatomists. A portion of Vesalius' (1514 - 1564) Book Five of De Corporis Humani Fabrica, published in 1543 concerns the urogenital system [3]. Within it there are four excellent illustrations of the kidney however, none shows another structure at the upper pole of either organ. This illustration is reproduced as Figure 1. Thus Vesalius, who became the father of human anatomy by demonstrating that the anatomic concepts of Galen (129 - 199) were derived from the dissection of animals, also seems to have overlooked the adrenal glands. So did the numerous anatomists who sought to confirm Vesalius' findings or to deny them, insisting that Galen was infallible.

It was not until 1564, over 20 years after publication of the Fabrica, that Bartolomeo Eustachius (1520 - 1574) first described the 'glandulae quae renibus incumbentes' [4]. The importance of the adrenal gland was then once again neglected until the father and son anatomists Caspar Bartholin (1585 - 1629) and Thomas Bartholin (1616 - 1680) turned their attention to the gland. Bartholin the younger described the gland as having no duct but containing a large cavity filled with a dark fluid. Although he confessed ignorance of the gland's true function, he speculated that the dark fluid in the cavity was bile - like liquid excrement derived mainly from blood that had passed through the spleen. The fluid accumulated until pressure forced it through the kidney producing the dark urine that often heralds the onset of disease. He named the glands 'capsulae atrabiliaria', a name that, among others, was in use until well into the 19th Century. The modern name, the 'suprarenal gland,' is attributed to Riolan the Younger (1580 - 1657).

Q,Q His characteribus ſiniſtræ lateris membrana notatur, quæ illi correſpondet, quam ſuper O, O indicarunt.

R,S Vteri ceruicis anterior pars, inter R & S ea adhuc obducta tunica, quam peritonæi partes illi offerũt, quæ ipſi uaſa exporrigunt, deducunt q;, ac illum peritonæo adnectunt. Cæterùm inter uallum inter R & S conſiſtens, uteri ceruicis amplitudinem quodammodo ſignificat. Rugæ uerò hic conſpicuæ, illæ ſunt quas uteri ceruix in ſe collapſa, neq; aliàs diſtenta, inter ſecandum commonſtrat.

T Veſica, cuius poſterior facies hic potiſsimum ſpectatur. ita enim in figuræ huius delineatione oculum direximus, ac ſi in corpore proſtrato, poſteriorem ueſicæ ſedem quæ uterum ſpectat, potiſsimum cernere uoluiſſemus. Si enim præſens muliebre corpus ita uti id quod modò ſubſequetur, erectum arbitrareris, etiam ſecus atq; res ſe habet, uteri fundum multo elatius ipſa ueſica delineatum eſſe tibi perſuaderes.

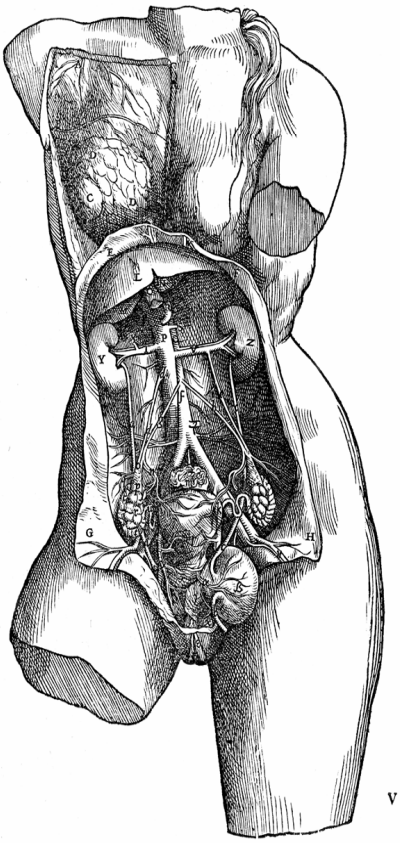
V Vmbilici eſt portio, à peritonæo inter ſecãdum liberata, & unà cum uaſis fœtui peculiaribus hic deorſum reflexa.

X Portio uenæ ab umbilico iecur petentis.

Y Meatus à ueſicæ fundi elatiſsima ſede ad umbilicum pertinens, ac fœtus urinam inter ſecundum & intimum ipſius inuolucrum deducens.

Z, et & Duæ arteriæ ab umbilico huc ſecundùm ueſicæ latera prorepentes, atq; hac ſede magnæ arteriæ ramis pubis oſsium foramina potiſsimum adeuntibus inſertæ, ſeu continuæ.

VIGESIMAQVINTA QVINTI LIBRI FIGVRA.



VIGE

Figure 1 Illustration of the Female Abdomen.

From reference 3 – note the absence of identifiable adrenal glands.

It was in 1855 that Thomas Addison (1795 - 1860) first made the connection between the clinical syndrome that consisted of hyperpigmentation and wasting with insufficiency of the adrenal gland. In 1855 Addison wrote a paper entitled 'On the Constitutional and Local Effects of Disease of the Suprarenal Capsules'. In his classic description, Addison described 10 cases characterised by 'anaemia . . . feebleness of the heart action . . . a peculiar change of colour in the skin occurring in connection with a diseased condition of the 'suprarenal capsules' [5]. The same paper also described pernicious anaemia, and it has been recognised as perhaps the only paper that describes two diseases, both named for the author [6].

The importance of the glands for normal physiological functioning was established unequivocally by Brown-Sequard (1817 - 1894), who proved that the glands were essential for life and that bilateral adrenalectomy was invariably quickly fatal [7].

It was in 1901 that Takamini first isolated adrenaline [8]. By 1929 investigators had discovered the gland was divided into two parts – the medulla that produced the vasoactive catacholamines, and the cortex that was responsible for the production of hormones that regulated sugar, salt and water balance [9].

It was not until the middle of the 20th Century and the Nobel Prize winning work of Kendall, Sarett and Reichstein that led to the discovery and synthesis of cortisone which provided the life saving glucocorticoid replacement therapy for hypoadrenal individuals [10].

Addison's Disease

Addison's disease, or chronic primary adrenal insufficiency is a relatively rare condition with a prevalence of 93 to 140 per million, and an incidence of 4.7 to 6.2 per million in white populations [11]. Women are more commonly affected than men with a male-to-female ratio of 1:1.5 - 3.5. The peak age of onset is in the 4th decade of life. It results from progressive destruction of the adrenals, which must involve > 90% of the glands before adrenal insufficiency appears. There are three broad categories of causes for non - iatrogenic hypoadrenalism. These are a) adrenal dysgenesis, b) adrenal destruction, and c) impaired steroidogenesis. These are more fully discussed elsewhere [11;12]. The most common cause in the developed world is probably idiopathic autoimmune destruction, with tuberculosis being possibly the most common worldwide cause of primary adrenal insufficiency. Other causes include adrenoleukodystrophy [13], bilateral haemorrhage [14], tumour metastases [15], HIV [16], histoplasmosis [17], cytomegalovirus (usually with HIV infection in the form of CMV necrotizing adrenalitis) [18], other infections due to the presence of HIV include *Cryptococcus neoformans, Toxoplasma gondii*, or Kaposi sarcoma [19], adrenomyeloneuropathy [13] or, very rarely, sarcoidosis [20].

Addison's disease is characterised by both glucocorticoid and mineralocorticoid deficiency, which require lifelong oral replacement therapy. Secondary adrenal insufficiency is most often caused by withdrawal of iatrogenic long - term glucocorticoid replacement, or by lesions within the hypothalamus or pituitary gland. In these conditions, while glucocorticoid function may be lost, mineralocorticoid function is preserved. This is because the trophic effects of ACTH are important in the maintenance of zona reticularis and zona fasciculata. Once ACTH drive is lost, either by pituitary

suppression by administration of exogenous steroids, or by primary or secondary hypopituitarism, the zona reticularis and zona fasciculata involute, leading to secondary adrenal insufficiency with preservation of mineralocorticoid function.

There are very few current estimates for the rate of death due to undiagnosed hypoadrenalism or for mortality rates in subjects with confirmed Addison's disease. When tuberculosis was more prevalent, the death rate was approximately 1.4 deaths per 1,000,000 cases per year.

At present the current replacement regime for people who are hypoadrenal consists of glucocorticoid and mineralocorticoid replacement. Hydrocortisone is the mainstay of glucocorticoid replacement therapy with alternative drugs being prednisolone, prednisone or, less commonly, dexamethasone. The dose of hydrocortisone for most adults (depending on their size) is a total of 20 mg to 30 mg per day. To simulate the normal diurnal adrenal rhythm the dose is divided throughout the day with most being given in the morning. Since this replacement dosage of hydrocortisone does not replace the mineralocorticoid component of the adrenal hormones, mineralocorticoid supplementation is usually needed. This is accomplished by oral administration of 0.05 mg to 0.1 mg of fludrocortisone per day. These replacement regimes restore life expectancy to that seen in subjects with normal adrenal function. However, despite normal biochemical values, overall quality of life in subjects with Addison's disease has been shown to be reduced [21-23].

Dehydroepiandrosterone and Dehydroepiandrosterone Sulphate

Biochemistry and Physiology of DHEA

Dehydroepiandrosterone (DHEA) [3β-hydroxy-5-androsten-17-one] and its sulphated ester (DHEAS) are two of the major C_{19} steroids secreted by the (innermost) zona reticularis region of the adrenals. Secretion is partly under the influence of adrenocorticotrophic hormone (ACTH) [24;25], but as levels of DHEA change during different phases of life without corresponding changes in circulating ACTH levels, this suggests that other factors determine DHEA release [26].

DHEA was first described in 1934 [27], and isolated 20 years later [28]. It was in 1960 that Baulieu showed that DHEAS was the most common form of the hormone found in the circulation [29].

As with all C_{19} steroids, these hormones are products of cholesterol metabolism and are derived from the action of the side chain cleavage enzyme product of the CYP11A1 gene (cytochrome P450scc) on the inner membrane of the highly active mitochondria found in the adrenal cortex [30]. DHEA and DHEAS are the most abundant circulating steroid hormones in humans. Their role remains to be fully elucidated. DHEAS can be readily converted to unconjugated DHEA by ubiquitous tissue steroid sulphatases, and thus probably serves as a reservoir for DHEA. DHEA is a weak, 17-ketosteroid group androgen precursor. Figure 2 shows a simplified illustration of the steroid synthesis pathway.

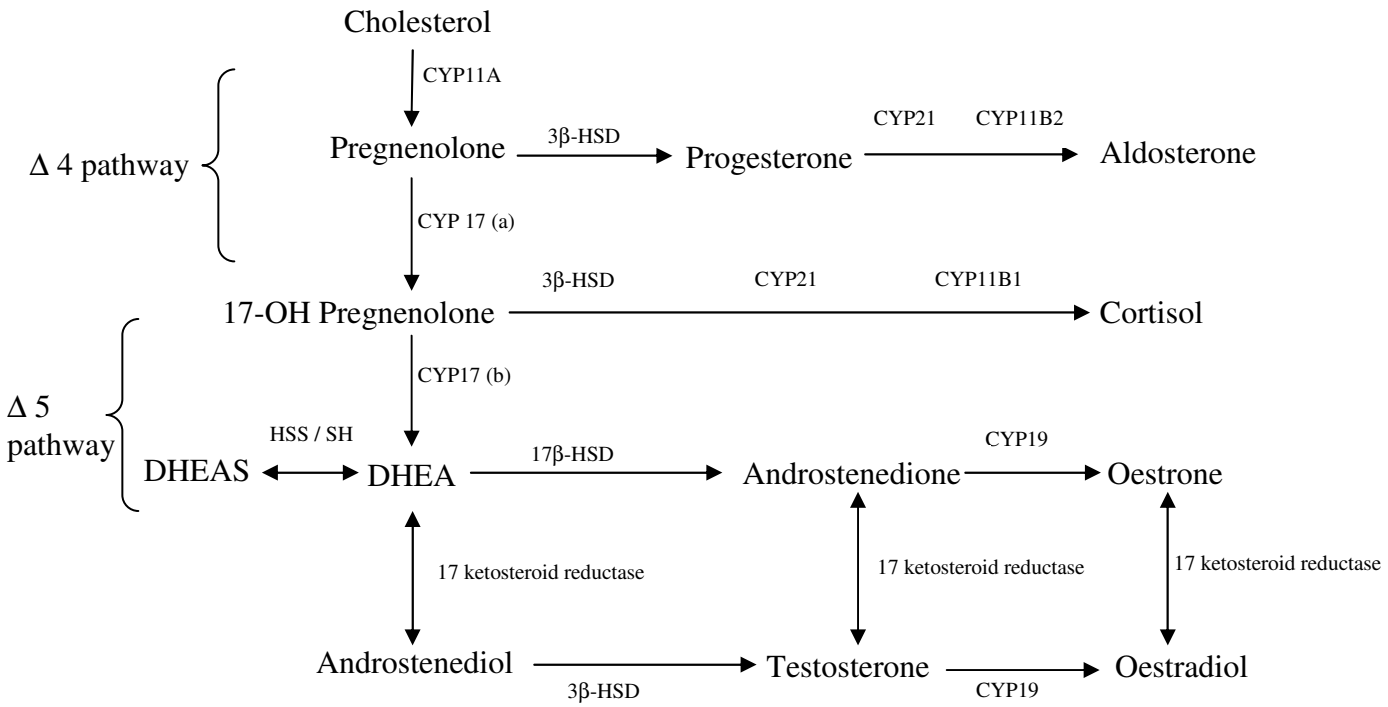


Figure 2 Simplified Steroid Synthesis Pathway

CYP 11A: 20, 22 Hydroxylase, 20, 22 - Desmolase; CYP11B1: 11β Hydroxylase; CYP11B2: 11β Hydroxylase, 18 - Hydroxylase and 18 - Oxidase; CYP17 (a): 17α Hydroxylase (catalysed by P450 $_{c17}$); CYP17 (b): 17, 20 Lyase (catalysed by P450 $_{c17}$); CYP19: Aromatase; CYP21: 21 Hydroxylase; 3β - HSD: 3β - Hydroxysteroid dehydrogenase; 17β - HSD: 17β - Hydroxysteroid dehydrogenase; HSS: 3β Hydroxysteroid sulphotransferase: SH: Sulphohydrolayse.

It can be seen that DHEA, testosterone, and oestrogens are derived from the precursor 17 - hydroxypregnenolone by the action of 17, 20 lyase, a reaction that is catalysed by cytochrome $P450_{c17}$. Thus, the adrenally secreted DHEA and DHEAS are sex steroid precursors. DHEA is the active form, with DHEAS being converted into DHEA by enzymes present intracellularly within the peripheral tissues, i.e. in an intracrine fashion [31]. This conversion is discussed below in greater detail.

While both DHEA and DHEAS are bound to albumin in the plasma, DHEAS is bound more firmly, and unlike DHEA, DHEAS is not bound to sex hormone binding globulin. In addition, because of the relative lack of protein binding, DHEA is rapidly cleared from the circulation - at approximately 2 litres per day - and has a half-life of 1 to 3 hours. For certain periods during life, due to it's short circulating half life, DHEA also has a circadian rhythm more closely related to that of the secretion of ACTH than DHEAS [25;32]. DHEAS is cleared at only 13 ml/day and subsequently has a half-life of 10 to 20 hours, thus levels do not vary greatly in the plasma [33]. These differences in clearance rates result in plasma DHEAS concentrations 250 to 500 times greater than DHEA [34].

A single and multi-dose study looking at the pharmacokinetics of DHEA showed that this hormone is handled differently in men and women [35]. This study looked at 6 men and 7 women aged between 64 and 79 years old given either placebo or 200 mg of DHEA for 2 weeks each. Blood levels of DHEA and DHEAS were checked several times over the course of the first day and again on day 15. This study showed that the half-life of DHEA in women was 11.7 hours, and in men 7.2 hours after the first dose, and then after 2 weeks of oral administration, this changed to 8.6 and 6.0 hours respectively. The half-

life for DHEAS was 27.1 and 25 hours for day 1 in men and women, and 23.8 and 20.8 hours respectively by day 15 [35].

The effects of DHEA(S) are thought to be mediated through a specific G protein coupled plasma membrane receptor [36], and through a specific nuclear DHEA receptor binding complex [37]. In much of the published literature DHEA and DHEAS are referred to as 'weak androgens', however there is no evidence that they bind to the androgen receptor. Thus, DHEA and DHEAS have little or no intrinsic androgenic activity. However, these hormones are converted into androstenedione and then further into potent androgens and oestrogens in the liver and other target organs [38]. These transformations are dependent on the tissue activity of steroidogenic and metabolising enzymes such as 3β - hydroxysteroid dehydrogenase/Δ^5 - Δ^4 - isomerase, 17β - hydroxysteroid dehydrogenase, 5α - reductase, and aromatase [39].

As can be seen from Figure 3, DHEA(S) levels vary profoundly throughout life in both sexes. Due to the immaturity of the 11β hydroxylase enzyme in utero, the Δ 4 pathway within the adrenal gland remains inactive, thus driving the cholesterol metabolites towards the Δ 5 pathway, resulting in high DHEA levels. As the 11β hydroxylase enzyme matures after birth, the zona glomerulosa become more active, reducing the activity of the Δ 5 pathway, thus lowering DHEA levels. Levels of DHEA then start to rise at 'adrenarche', i.e. between 8 to 10 years of age, reaching a peak by the middle or end of the second decade of life. Levels then decline by 10% per decade plateauing after the age of 80 [40;41]. Although there are a number of theories why this decline occurs, the reason currently remains unknown [42]. It has been proposed that one of the potential causes of this reduction of DHEA(S) levels is due to the reduction in

enzyme activity of 17, 20 lyase that converts 17 hydroxypregnenolone to DHEA resulting in low levels of DHEA and testosterone [42-44]. This is manifested in the elderly by a reduced level in circulating DHEA(S) levels and by a blunted response to ACTH stimulation, with a normal cortisol response [45]. However, the picture becomes more unclear because in conditions where there is chronic ACTH stimulation, DHEA(S) levels may remain normal or decrease [46]. In health, whilst cortisol is under hypothalamic – pituitary control via a negative feedback mechanism, DHEA secretion is not [46]. This is illustrated in Figure

4

Figure 3 Changes in Levels of Circulating DHEAS with Ageing

From reference 40.

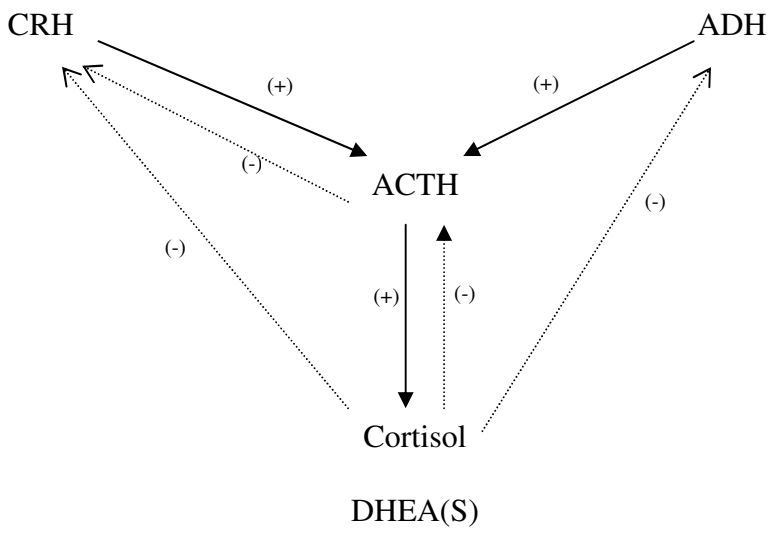


Figure 4 The Hypothalamic – Pituitary – Adrenal Axis

The solid arrows with black arrowheads represent stimulation (+), the dotted arrows with open arrowheads represent inhibition (-). Corticotrophin releasing hormone (CRH), and antidiuretic hormone (ADH) from the hypothalamus, stimulate adrenocorticotrophic hormone (ACTH) release from the anterior pituitary, which in turn stimulated the adrenals to produce both cortisol and DHEA(S). However, only cortisol feeds back to inhibit ACTH, CRH and ADH. Adapted from reference 46.

It has been suggested that circulating plasma DHEA(S) levels are a marker for longevity [1]. Further evidence for this comes from looking at ethnic differences in DHEA(S) levels which suggests that life expectancy may be higher in those populations in whom DHEA(S) levels are highest [47].

It remains to be determined if DHEA per se has any intrinsic hormonal activity. The effects of DHEA are due to the actions of the sex hormones into which it is converted. While recent studies have found specific receptors to help explain some of the actions of DHEA, the mechanisms of other actions of this hormone remain elusive.

Examples of these receptors include skeletal muscle binding sites [48]. These receptors may be of therapeutic value in the treatment of disorders associated with low DHEA levels, such as myotonic dystrophy [48]. Other receptors include those thought to be responsible for some of the protective cardiovascular effects of DHEA [36;49].

These potentially beneficial cardiovascular effects are mediated through a variety of possible mechanisms. One is thought to be a specific G protein coupled plasma membrane receptor leading to an increase in endothelial nitric oxide synthase [36]. Other recent animal work has suggested that there are unique mechanisms within vascular endothelial cells that account for the DHEA induced restoration of nitric oxide levels by enhancing and stabilising endothelial nitric oxide synthase expression. This appears to be by direct effects of DHEA on the genome, but also by non-transcriptional mechanisms [49]. Another DHEA specific receptor-binding complex has also been found in murine and human T cells. In this in vitro model using intact murine T cells, DHEA binding led to an increase in interleukin 2 production leading to the claim that DHEA 'improves immune function' [37;50]. However, the mechanism of action of DHEA on this receptor has yet to be described.

The decline in circulating DHEA levels parallel many age-related changes such as sarcopaenia and osteopaenia. In men, studies with castrated subjects show that approximately 30% to 50% of circulating androgens are derived from DHEA. The remaining androgens come from the testes as testosterone. Both DHEA and testosterone are then converted to the active androgen dihydrotestosterone, in the peripheral tissues [51]. In postmenopausal women however, there is conflict over the origin of most circulating androgens. Some studies have shown that these androgens are derived from DHEA, with

less than 50% coming from the ovaries [52;53], whilst other work has shown that the ovaries remain an important source of testosterone [54;55].

Whatever the origin of these androgens, women with adrenal insufficiency suffer from chronic DHEA(S) deficiency, because routine replacement therapy with glucocorticoids and mineralocorticoids fails to restore the androgens derived from the adrenal precursors. Replacement of adrenal precursor derived androgens in subjects with adrenal insufficiency should ideally restore DHEA(S) concentrations to levels equivalent to those before the onset of the condition. There has been a great deal of previous work to determine the optimal replacement dose in both elderly [56], and hypoadrenal [57] subjects to restore DHEA(S) levels to that seen in young adults. This has been shown to be 50 mg per day, which is sufficient to increase DHEA(S) levels into the normal range found in young adults [58;59].

Most previous studies have investigated changes in physiological parameters in DHEA(S) deficient individuals, both healthy elderly and hypoadrenal. Clearly there are differences to be expected between the normal physiological process of ageing, and the pathological state of adrenal insufficiency. In hypoadrenal individuals there is little or no circulating DHEA, the elderly however, whilst having substantially lower levels than healthy individuals in their second or third decade, have levels that may be several fold greater than the hypoadrenal individual. These differences mean that relating and inferring results from one group to another is more difficult; however, the premise of much of the current clinical research in both ageing and hypoadrenal subjects is that the two are interchangeable. This link between the elderly and hypoadrenal subjects is compounded by the findings of studies such as those looking at different measures of

general well being, libido and mood that have found similar results in both groups of subjects [21;22;58;60].

Many of the disorders of ageing such as reduced immunocompetence, obesity, diabetes and cancers have been attributed to changes in DHEA(S) on the basis of animal studies [61;62] and epidemiological data [63-66]. Further animal studies in New Zealand white rabbits have shown potential cardiovascular benefits, including a reduction in cholesterol accumulation within the large vessels [67;68], and an in vitro human model has shown inhibition of platelet aggregation [69]. Despite these findings, there is conflicting evidence from epidemiological human studies as to whether circulating androgen levels are associated with changes in cardiovascular mortality [64;70].

Other work has been done looking at the effect of DHEA supplementation in autoimmune conditions, with some success [71]. This study looked at 191 women with systemic lupus erythematosus on corticosteroids. Subjects were randomised to placebo, 100 mg, or 200 mg of DHEA daily for 7 to 9 months. At the end of this time, there was a statistically significant reduction in corticosteroid use without a corresponding increase in symptom scores, or laboratory indices of disease activity in those subjects treated with 200 mg of DHEA compared with those on placebo.

In addition to these findings, a relative or absolute lack of DHEA(S) has been associated with effects on muscle tissue that include a decrease in muscle strength, fibre size and number [72;73]. However, data showing that these changes can be reversed in either hypoadrenal subjects or normal elderly subjects by restoring DHEA(S) levels has been conflicting to date [22;74;75].

DHEA(S) replacement therapy may be of special importance for patients with adrenal insufficiency, because adrenal androgen precursor deficiency in these patients is frequently neglected [76]. Accordingly, it has been shown that despite an otherwise adequate glucocorticoid and mineralocorticoid replacement regime in Addison's disease or hypoadrenalism, quality of life may be inferior to that of subjects with functioning adrenal glands [23;77]. Thus, DHEA replacement in subjects with adrenal insufficiency may hold the potential to improve both their well being and their functional status [78]. Moreover, DHEA administration to these patients is well suited to gain further insight into the psychological and physiological role of DHEA, because a true deficiency is replaced. The issues regarding of the effects of DHEA replacement on the mood and well being of hypoadrenal women are discussed elsewhere, as part of the larger study from which the present data is derived [79].

Relatively few studies have looked specifically at the use of DHEA(S) replacement in hypoadrenal and adrenalectomised subjects. Those studies available, have shown conflicting results [21;60;75;80]. In addition, previous methods of analysis have proved unsatisfactory in many instances, e.g. the use of only bioelectrical impedance and waist hip ratios for anthropomorphic measurements, or the use of fasting glucose and insulin alone to assess insulin sensitivity [75].

The Effects of DHEA on Insulin Sensitivity

Most of the work done in this area has been in animal models. It is more difficult to extrapolate these results to human studies, because as mentioned, rodents do not produce any endogenous DHEA, and the demonstrated effects are pharmacological rather then physiological. However, there are some human studies and these will be reviewed in this section. The relevant in vitro and animal studies are also discussed.

Animal studies have shown that DHEA administration increases insulin sensitivity [81-83]. DHEA administration lowers serum insulin in fatty Zucker rats [84;85]. Coleman et al reported that DHEA prevented the onset of diabetes in *db/db* mice [61]. Why this occurred was unclear because the reduction in blood glucose was achieved without improvement of insulin resistance in peripheral tissues and without lowering pancreatic insulin content.

Insulin has been positively correlated to metabolic efficiency [86], i.e. efficiency of body weight gain, and thus implicated as a causative factor in the aetiology of obesity. The 40% drop in insulin levels with DHEA supplementation may be a contributing factor in lowering body weight gain or altering metabolic efficiency in DHEA treated rats [84;85]. When comparing the growth of obese Zucker rats, DHEA supplementation caused weight loss in both castrated and noncastrated animals, suggesting that the antiobesity effect is either due to a direct effect of DHEA or due to DHEA derived androgens [87].

Lowered serum insulin levels may be responsible for lowered activities of lipoprotein lipase, glucose - 6 - phosphate dehydrogenase and fatty acid synthetase measured in DHEA treated obese rats [84]. However, the lack of effect of DHEA on the glucose tolerance test suggests no effect on peripheral glucose metabolism. This is

supported by other studies showing no effect of DHEA on glucose metabolism either in isolated adipocytes [88], or in soleus muscle [85]. The lack of effect on peripheral glucose metabolism is also supported by other studies showing no effect of DHEA on muscle GLUT 4 content [83]. Some effects on glucose metabolism have also been shown to occur in the liver, with DHEA treatment being associated with suppression of the gluconeogenic enzyme glucose - 6 - phosphatase in *db/db* mice [89].

These findings are in contrast to in vitro studies which have shown that DHEA(S) causes an increase in T lymphocyte insulin binding with a corresponding increase in pyruvate dehydrogenase enzyme activity [90]. This enzyme was chosen as it represents an accurate reflection of metabolic activities within other tissues such as skeletal muscle, adipose tissue, and the kidney. Pyruvate dehydrogenase in T lymphocytes reflects the degree of glucose intolerance of hyperandrogenic women and in diabetic subjects in whom enzyme activity is impaired [91]. The authors of this study do say that the evidence for DHEA infusion improving some aspects of insulin sensitivity in vitro may suggest that the 17 hours of infusion they gave to achieve a serum level of 2.5 times greater than baseline may not have been long enough to see a clinically significant change in insulin sensitivity in vivo.

There are also divergent effects on food intake in lean and obese rats given DHEA. In obese Zucker rats, DHEA reduced food intake, while in lean animals food intake was increased [84;92;93]. The mechanism for this difference is unknown, however DHEA has been shown to decrease leptin secretion in an animal model [94].

Previous work has looked at the effects of DHEA(S) in preventing complications of established diabetes. These were the vascular and neural dysfunction. DHEA treatment

resulted in some success [95]. In this study, Yorek et al used varying doses of DHEA in the diet of rats rendered diabetic with streptozotocin. In the control group motor nerve conduction velocity and endoneurial blood flow were significantly reduced, but this deterioration was prevented by DHEA supplementation over a 4 to 5 week period. These results are illustrated in Figures 5 and 6. These authors also showed that at a dose of 0.25%, DHEA significantly lowered the diabetes-induced increase in substances known to cause oxidative stress. DHEA also reduced the production of superoxide by epineurial arterioles of the sciatic nerve. In addition, 0.25% DHEA treatment significantly improved vascular relaxation mediated by acetylcholine in epineurial vessels of diabetic rats. These studies suggest that DHEA, by preventing oxidative stress and perhaps improving sciatic nerve Na^+-K^+-ATPase activity, may improve vascular and neural dysfunction in diabetes [95]. More recent work has complimented this by the use of subepineurial injections of DHEA in transected nerves. This has shown that DHEA reduced the atrophy usually associated with denervation, as well as speeded up neural regeneration [96].

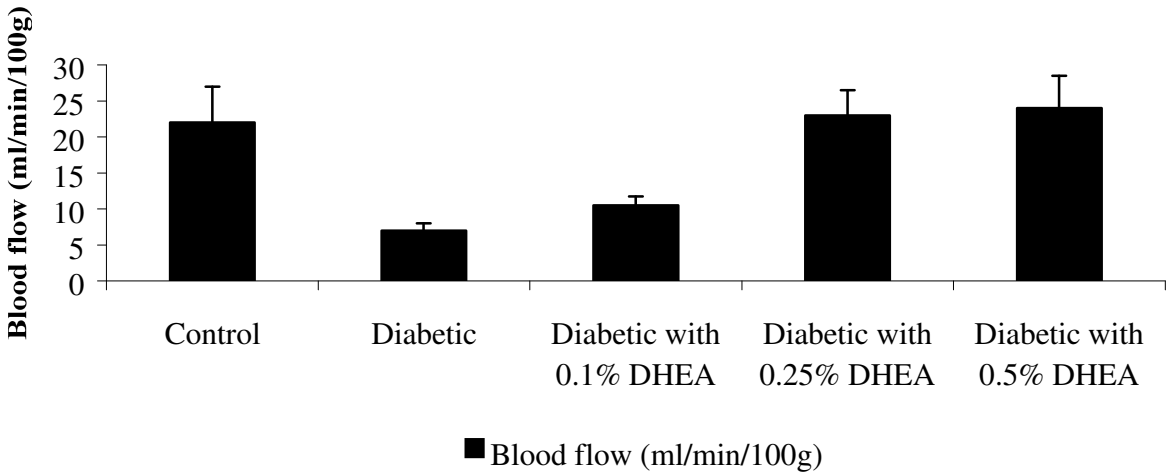


Figure 5 Endoneurial Blood Flow in Diabetic Rats With and Without DHEA Supplementation

From reference 93

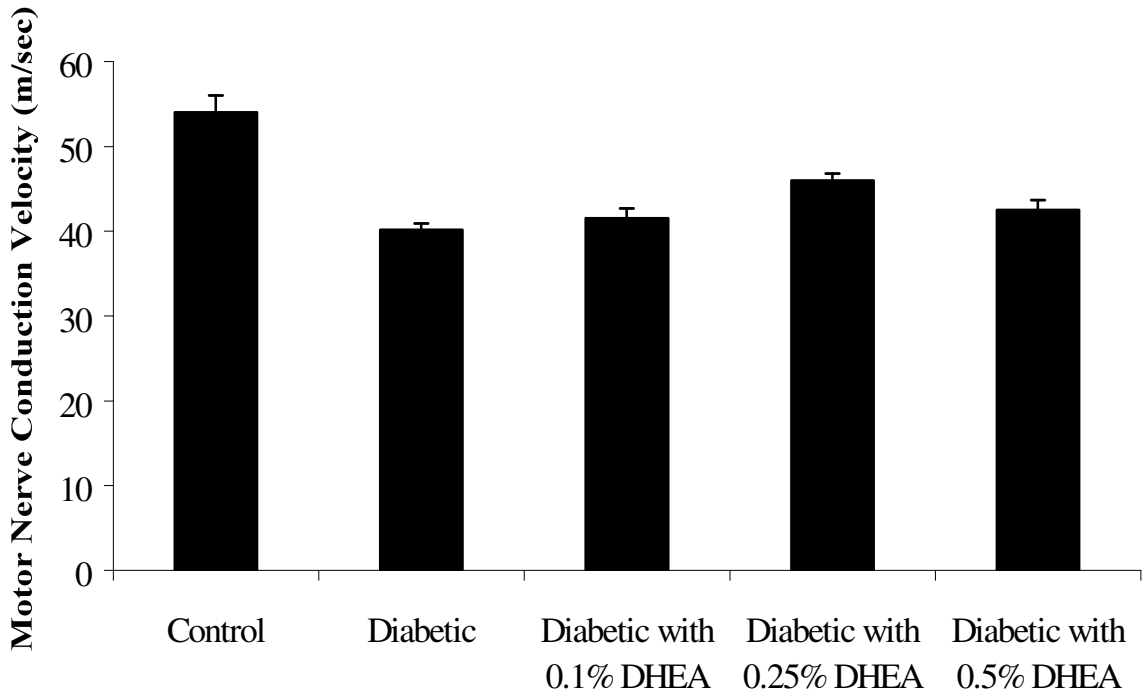


Figure 6 Motor Nerve Conduction Velocity in Diabetic Rats With and Without DHEA Supplementation

From reference 93

The Effects of DHEA on Muscle Protein Synthesis

Muscle Mass and Muscle Protein Dynamics

Morphological studies have provided no clues as to the biochemical basis for the declining muscle mass seen with ageing. Muscle cells are made up of mostly protein. These proteins are critical for muscle functions. The contractile function of muscle depends on the availability of several elements, of which myosin is a key contractile protein. The decline in muscle mass and muscle function with ageing indicates a reduction in muscle protein content. During growth, synthesis of muscle proteins exceeds muscle protein breakdown [97]. Once linear growth has ceased a continuous process of protein breakdown and protein synthesis (i.e. turnover) maintains the quality and quantity of muscle protein. These processes are regulated by gene expression that is modulated by various physiological and pathological factors such as exercise and nutrition [98].

A decline in muscle mass occurs only when muscle protein synthesis lags behind muscle protein breakdown [97;98]. However, whole body protein turnover studies are not sufficiently sensitive to detect the small changes that occur only in muscle because protein synthesis in muscle occurs at a slower rate than in other tissues such as liver and gut [97;99;100]. Whilst previous measurements of synthesis rates of mixed muscle protein and the myofibrillar component of mixed proteins have shown a decline in the elderly, there was no such change in the whole body protein turnover [100-102]. Other authors have taken whole body protein breakdown measurements using urinary 3-methylhistidine excretion to show that a decrease in whole body protein synthesis occurs with ageing. Furthermore, using methods to measure whole body protein turnover it has been shown that the decline in muscle mass is entirely due to a

decline in muscle protein synthesis rather than an increase in protein degradation [103;104].

None of the studies mentioned above measured the synthesis rate of specific muscle proteins directly. The myofibrillar component constitutes approximately 60% of total muscle protein, but the myofibrillar fraction is comprised of proteins such as myosin (which comprises of myosin heavy chain and myosin light chain), actin, fibre C-protein, X-protein, tropomyosin, troponin and nebulin. In addition, proteins from intermyofibrillar mitochondria may contribute to this fraction [105]. Different genes regulate each of these proteins and regulation of their synthesis rates is likely to be different. Therefore, to understand the cause of sarcopaenia in individuals who lack DHEA, such as the hypoadrenal or elderly, it is essential to understand the molecular mechanism(s) of the impairment in synthesis of these various constituent muscle proteins. This requires measurement of the synthesis rate of specific proteins.

Nair et al have developed isotopic techniques that enable measurement of the protein synthesis rate of myosin heavy chain isolated from skeletal muscle needle biopsy samples [106-108]. This approach was used to measure the synthesis rate of MHC in young (age 20 to 30 years), middle aged (45 to 56 years), and elderly (65 to 94 years) individuals. Paralleling what other authors have shown regarding the reduction in age related levels of circulating DHEA, Nair et al demonstrated a reduction in MHC synthesis rates with ageing. Specifically, a 31% decline in the synthesis of MHC from young to middle age, and a further 13% decline from middle to old age [103;104]. This decline was correlated with a reduction in muscle strength, although there was no correlation between muscle strength and synthesis rate of mixed muscle protein. These data imply that a decline in the synthesis rate of myosin heavy chain contributes to the reduction in the contractile function of skeletal muscle with ageing.

A direct comparison between MHC synthesis rates and DHEA levels has never previously been made.

Nair et al also purified mitochondria from skeletal muscle needle biopsy samples and measured its protein synthesis rate [104]. Mitochondrial protein synthesis rate in young people (0.082 ± 0.003 %/h) was approximately twice as high as the mixed muscle protein synthesis rate (0.041 ± 0.003 %/h) measured in the same subjects. Mitochondrial protein synthesis rate was 44% lower in the middle aged than in the young, with no further deterioration with advancing age [104]. In addition, it was demonstrated that ageing is associated with a progressive loss of mitochondrial content (as reflected by decreased maximal activities of two mitochondrial enzymes: citrate synthase and cytochrome c oxidase) within skeletal muscle. The decline in VO_2 max observed with advancing age might be explained at least in part on the basis of declining mitochondrial content.

The measurements made by Nair et al provided the first evidence to support a hypothesis for age-related sarcopaenia based on mitochondrial damage. Mitochondrial DNA damage by free radicals was proposed by Harman [109]. Based on the findings from Nair et al, it is possible that a decline in mitochondrial content resulting from decreased regeneration of mitochondrial protein (i.e. a reduced synthesis rate) could compromise the capacity of muscle to generate ATP. A decline in ATP production can cause not only fatigability of muscle, but might also deprive the cell of the necessary energy for protein synthesis, an energetically expensive process. A reduced ability to synthesise protein is reflected by a decline in fractional synthesis rate of myosin heavy chain, the pivotal contractile protein. A decline in the synthesis rate of myosin heavy chain with no evidence of myofibrillar protein breakdown could result in a progressive decline in myosin heavy chain content in muscle [101]. This progressive

decline of the important contractile proteins could cause a decline in muscle strength and other functions.

Another important observation was that, although synthesis rates of MHC and mitochondrial protein declined with ageing, there was no change in synthesis rate of sarcoplasmic protein, which is mainly responsible for anaerobic ATP production and ionic transport. This differential effect of age on synthesis rates of various muscle proteins could not be detected by measuring synthesis rates of mixed muscle protein and MHC.

It is not known if adrenal insufficiency has an effect on skeletal muscle protein fractional synthesis rates. The propose of part of the present study was to determine whether DHEA(S) replacement in hypoadrenal individuals on adequate replacement therapy altered MHC and mitochondrial protein synthesis rates.

Whole Body and Fractional Muscle Protein Synthesis Rates

The underlying mechanism of the effect of DHEA(S) on muscle metabolism is not clear. It is known that DHEA(S) serves as a precursor of other androgenic and oestrogenic products [31]. As mentioned previously, these products are believed to be produced in peripheral tissues, and may serve to supply local requirements under the regulation of a series of DHEA(S) metabolising enzymes [110]. Studies in humans indicate that DHEA administration increases circulating levels of androstenedione, as well as total and biologically active IGF 1 levels [60]. DHEA(S) has also been reported to increase insulin sensitivity in animals and humans [61;111]. IGF 1 has been shown to specifically stimulate muscle protein synthesis in humans [112], and insulin promotes muscle protein anabolism by inhibition of protein breakdown [113]. There is evidence that in certain in vitro models, individual cell lines respond to DHEA(S) as an

anabolic hormone. However, there are also conflicting reports suggesting that DHEA(S) delays or enhances apoptosis [114;115]. These effects have not been studied in vivo in animal models or humans.

The Effects of DHEA on Mitochondria

The mitochondrion is the only organelle in the cell, aside from the nucleus, that contains its own genome and genetic machinery. The human mitochondrial genome is a 16.6-kb circle of double-stranded mitochondrial DNA (mtDNA) [116]. MtDNA is made up of 24 genes that specify two ribosomal RNAs and 22 transfer RNAs that are required to synthesise the 13 polypeptides [117]. These 24 genes encode 13 polypeptides, all of which are components of the respiratory chain / oxidative phosporylation system. About 850 polypeptides, all encoded by nuclear DNA, are required to build and maintain a functioning organelle. These proteins are synthesised in the cytoplasm and are imported into the organelle, where they are partitioned into the mitochondrion's four main compartments - the outer mitochondrial membrane, the inner mitochondrial membrane, the intermembrane space, and the matrix, located in the interior. Of these 850 proteins, approximately 75 are structural components of the respiratory complexes and at least another 20 are required to assemble and maintain them in working order. Among these translational products of the mitochondrial genome are the five complexes of the respiratory chain / oxidative phosporylation system - complexes I (NADH ubiquinone oxidoreductase), II (succinate ubiquinone oxidoreductase), III (ubiquinone - cytochrome c reductase), IV (cytochrome c oxidase), and V (ATP synthase). All of these complexes are located in the inner mitochondrial matrix. There are also two electron carriers, ubiquinone (also called coenzyme Q) which is located in the inner mitochondrial matrix, and cytochrome c, located in the intermembrane space [117].

Translation of the mRNAs that encode these proteins within mitochondria leads to the formation of protein subunits that are vital for normal respiratory

function. Any problems within these complex pathways can lead to mitochondrial (and whole body) dysfunction – i.e. the mitochondrial myopathies.

DHEA administration is reported to have a thermogenic effect with enhancement of mitochondrial and cytosolic enzymes [118;119]. These studies were performed in rats and therefore need to be confirmed in humans since rats normally have very low or absent levels of DHEA(S), and the reported effects may thus be pharmacological.

Mitochondrial dysfunction increases in humans with ageing [120]. Whether this dysfunction is due to free radicals remains to be determined. This reduction in mitochondrial function parallels the decline seen in DHEA levels. It is a matter for debate over whether a 'mutation' equates to 'dysfunction', however the mutations found in mitochondria of ageing subjects are not usually seen in younger humans, and the mitochondria of ageing subjects have a lower capacity for normal function – e.g. ATP production, than younger individuals [121]. It has been suggested that this decline in mitochondrial function may have a threshold, below which defects in cellular oxidative phosphorylation occur. This effect can be seen by measuring the activity of a number of enzymes involved in the respiratory chain complexes, especially cytochrome c oxidase (COX) [122]. Mutations resulting in dysfunction within individual cells may not be important, unless the cells were an integral part of a complex system.

There has been early work to suggest that mitochondrial mutations are associated with tumourigenesis, with a higher number of mutations being associated with neoplastic tissue than is found in normal tissue within the same person [123]. A recent hypothesis has been put forward to unify the observations in mitochondrial damage seen in ageing, cancer and specific mitochondrial diseases [124]. These authors suggest that there are three basic reasons why these effects are seen – mitochondrial

polyploidy, relaxed replication, and a mechanism that regulates normal mitochondrial copy numbers. These account for the normal genetic drift seen in replicating cells, but that in disease these changes may occur at a faster rate, leading to a derangement of these regulatory mechanisms.

Early work suggested DHEA administration to rodents in highly suprapharmacological doses (50 mg/Kg) lead to a marked decline in oxygen consumption in the mitochondria of a variety of tissues including heart, kidneys, adrenals, brain and brown adipose tissue [125;126]. However the effect on the heart in vivo was only observed for the first few hours after intraperitoneal injection following which the effect declined. The effect was not observed if the DHEA was given orally. It may also be that the mitochondrial effects of DHEA(S) are not limited to oxygen consumption and respiration because orally administered DHEA has also been shown to cause a marked reduction in carbomyl phosphate synthetase - 1, in mice and rats [127]. This is a key enzyme that comprises up to 15% to 20% of mitochondrial matrix and is the rate-limiting enzyme in the urea cycle. Despite this reduction in enzyme production, urea levels remained normal suggesting that the enzyme is working below its V_{max}.

Whilst these findings are potentially of interest and may provide clues to some of the biological functions of DHEA(S), these studies are clearly highly artificial for two reasons: a) rodents produce virtually no endogenous DHEA(S) and b) the doses used are highly suprapharmacological. Indeed, at these very high doses, one study found that immature female Sprague – Dawley rats were induced into anovulatory cycles with the formation of follicular cysts to give a condition very similar to that seen in polycystic ovarian syndrome [128]. This suggests that sexual maturity can be partially induced by the production of sex steroids induced by large doses of DHEA in

rodents. It remains a matter of debate if the increase in cholesterol synthesis [129], or the conversion of cholesterol to DHEA (and subsequently to the various sex hormones) is the cause of, or occurs as a result of, the onset of puberty [130]. The dose used in the present study in humans was approximately 150 to 200 times less than the dose given by Anderson et al [128] to the rodents on a per kilogram basis. The dose chosen in the present study was based on previous work looking at optimal doses required to restore plasma DHEA(S) levels to that seen in young healthy subjects [57;59].

Hepatic mitochondrial proliferation occurs with DHEA administration and elevated rates of mitochondrial respiration have been measured [131]. These rates may be elevated by a variety of mechanisms including the induction of a futile / substrate cycle of fatty acid deacylation / reacylation or by increasing the activities of cytosolic enzymes involved in NADPH production (such as malic enzyme, isocitrate dehydrogenase and aconitase) which increase within a few days after DHEA(S) administration [132;133]. This is in conflict with other data from Wu, who showed that the enzymes involved in NADPH production were inhibited by DHEA in vitro [134]. That study using a porcine cell model showed NADPH production was reduced by inhibiting the activity of several enzymes of the pentose cycle, in particular glucose - 6 - phosphate dehydrogenase. Thermogenesis has also been proposed to occur by the increased uncoupling protein induced proton leak that may be the mechanism for the physiological findings seen in hyperthyroidism [135;136]. DHEA induced uncoupling has recently been shown to occur in brown adipose tissue of rats [137]. There is also some data to suggest that DHEA potentiates ATPase gene expression [138].

In summary, there appear to be a number of different mechanisms which may increase either futile ATP turnover or thermogenesis which ultimately may be one of

the explanations for the weight loss that is occasionally seen in DHEA(S) treated rats that loose weight despite no decrease in food intake.

The Effects of DHEA on Peroxisomes

Work looking at the effects of DHEA(S) on peroxisomes has shown a possible mechanism for the beneficial effects seen with administration of this drug to rodents. It has been postulated that the decline in immune function seen in ageing is due in part to a reduction in size and activity of peroxisomes [139]. Work by Frenkel et al has shown that in rodents DHEA(S) caused both a change in mitochondrial numbers and an increase in mitochondrial size [140]. They also showed that DHEA(S) caused a proliferation in peroxisomal numbers and increased activity of a number of peroxisomal enzymes. This was later confirmed by Mastrocola et al, who showed that at high doses DHEA induced hydrogen peroxide levels while reducing the levels of intracellular antioxidant reduced glutathinone. This did not occur when DHEA was given at lower doses suggesting a dose related effect on pro-oxidant / antioxidant activity [141]. Mohan and Cleary also demonstrated that both hepatic hydrolase and catalase activities were increased after 24 hours of DHEA administration, so leading to increased peroxisomal beta-oxidation rates [131]. This was later confirmed in a human hepatic cell line, however, the effects seen on peroxisomal enzyme activities were not as dramatic as those in the rodent model [142].

As peroxisomes are essential to normal immune function it may be inferred that the decline in incidence in some conditions related to normal ageing seen in rodents given DHEA(S) may be due to this effect. Some caution with this assumption is necessary however, as it is well described that prolonged courses of high doses of DHEA(S) in rodents leads to the development of hepatocellular carcinoma. In one particular study looking at the origin of this tumour in rats treated with DHEA(S) for up to 84 weeks, it was found that the initial lesions in the liver were found after 32 weeks and that these lesions were highly associated with perivenular hepatocytes in

which both mitochondrial and peroxisomal proliferation predominated [143]. So whilst mitochondrial and peroxisomal proliferation may have some initial beneficial effects, this may not be the case in the long run.

In looking to try and prevent the detrimental effects of excessive DHEA(S), antioxidant therapy with α tocopherol has been tried. This resulted in a decline in the DHEA induced increase in lipid peroxidation [144]. This may suggest a role for antioxidants in the DHEA(S) induced liver damage, but as this very rarely occurs at the doses used in human studies it is unlikely to be clinically useful. In addition, as mentioned previously, this was in rodents, who have no endogenous production of DHEA(S), and in very large doses. The extrapolation of this finding to humans is therefore a difficult one to make.

The Effects of DHEA on Insulin Like Growth Factors and Their Binding Proteins

IGF 1

Insulin like growth factor 1 is a single chain polypeptide with 70 amino acids and is homologous to both IGF 2 and proinsulin. The basic peptide is a product of a single copy gene located on the long arm of chromosome 12 [145]. IGF 1 is important for a wide range of cellular functions, including proliferation, differentiation, cell function, and cell survival [146]. Levels of IGF 1 are age dependent, with a peak at puberty followed by a steady decline with ageing [147]. Because of this, girls peak at an earlier age than boys do, although the rate of decline is then equal in both sexes [148]. Only a very small quantity (less than 1%) of IGF 1 is found circulating free in the circulation. Of the remainder, 80% is bound to IGF BP 3, with the rest bound to one of the other IGF BP's. Conflicting evidence exists, suggesting that the genetic influence on plasma levels of IGF 1 levels ranges from 38% to 63% [149;150].

IGF 2

Insulin like growth factor 2 is a single chain 67 amino acid protein that is the product of a single gene found on the short arm of chromosome 11 [151]. There is a significant genetic component with up to 66% of plasma levels being attributable to heritability [150]. IGF 2 levels vary little with gender and peak at puberty followed by a slow decline with ageing, plasma levels being only 20% to 30% lower in the elderly compared with young adults [148;152]. It had not previously been determined what IGF 2

levels are in hypoadrenal subjects. Skeletal muscle mRNA levels of either IGF 1 or 2 and IGF BP4 and IGF BP5 have also not been measured.

IGFBP's

The insulin like growth factor binding proteins are a group of six known structurally homologous proteins. Whilst they are transport proteins, their primary function is to prolong the half-life of circulating IGF 1 and IGF 2. IGF BP3 is particularly important in this respect. Elongation of circulating half-life is done by the binding of IGF to the IGF BP3. Together they form a stable tertiary complex with the acid-labile subunit found circulating in the plasma [153]. The formation of this complex stabilises the IGF's and so prolongs the biological half-life [154]. This ensures a steady supply of IGF 1 to the tissues and also modulates the endocrine and paracrine actions of the IGF's. This binding also influences the bioavailability and facilitates storage of the IGF's in the extracellular fluid.

IGF BP1

Insulin like growth factor binding protein 1 is a nonglycosylated protein encoded by a single copy gene located on the short arm of chromosome 7, only 20 kilobases away from the gene that codes for IGF BP3 [155]. Heritability accounts for only 36% of circulating levels [149], however there remains some discrepancy and debate about this, as IGF BP1 is the only binding protein in this group to show a substantial diurnal variation in plasma levels [150]. This is important because both the metabolic regulation of, and adaptation to, nutritional intake are associated with changes in circulating concentrations

of serum glucose and insulin levels [156;157]. Insulin has been shown to inhibit hepatic production of IGF BP1 [158]. This was later confirmed in a study looking at IGF BP1 levels in obese subjects with hyperinsulinaemia [159]. These authors showed that IGF BP1 levels were significantly lower than normal weight controls, and that free IGF 1 was therefore elevated in these subjects whilst total IGF 1 remained unchanged compared to controls.

IGF BP3

Insulin like growth factor binding protein 3 is a glycoprotein encoded by a gene close to that encoding IGF BP1, located on the short arm of chromosome 7. This binding protein is the main carrier of IGFs in serum and forms a circulating complex with both IGF 1 and the acid-labile subunit. Up to 60% of circulating levels can be attributed to genetic factors [150]. Levels of both IGF BP3 and IGF 1 reflect endogenous GH secretion [160], and are subsequently both low in GH deficient adults [161]. Ageing is associated with a decline in the level of this protein similar in fashion but not as dramatic as the decline seen with DHEA(S). As with DHEA(S), levels peak at or just after puberty with girls having slightly higher plasma levels than boys [148]. It is not known if the decline is DHEA(S) related. Several studies have shown that levels remain unchanged with DHEA administration [58;60;162].

Whilst there has been work looking at the effect of IGF 1 and 2 on DHEA production in adrenal cell cultures [163], there has been little in vivo work looking at the effect of DHEA(S) on either serum IGF levels or IGF BP mRNA expression rates. Ageing and wasting syndromes are associated with a decline in circulating IGF 1 and a rise in IGF BP1. In healthy elderly subjects, it has been demonstrated that DHEA

supplementation increases serum IGF 1 levels with a corresponding decrease in IGF BP1 [164;165]. This was achieved without affecting growth hormone secretion rates [166]. In addition, higher levels of DHEA and IGF 1 levels are positively correlated with physical activity in elderly men [167].

Exercise increases DHEA and GH levels in healthy individuals. As shown in Figure 4, DHEA may rise due to the influence of ACTH, but this has not been fully explored. Serum levels of binding proteins change after exercise in humans [168], and animal studies have demonstrated that exercise can increase skeletal muscle IGF BP4 mRNA levels and decrease IGF BP5 mRNA levels [169]. It is unknown if these effects are due to the exercise itself or to the hormonal response to the exercise. It has been shown in rats that are exercised for 5 days, skeletal IGF 1 rises significantly, whilst IGF 1 mRNA remains unchanged [170]. These authors raise possible explanations for this finding such as there may be translational or post-translational modifications to increase intramuscular IGF 1. It could also be that other, non-genetic, mechanisms may cause this increase, such as an increase in intracellular binding protein levels, or increases in muscle blood flow. The present study was designed to allow an initial assessment of these proteins in hypoadrenal subjects and also an assessment of the effects of DHEA replacement on them by mimicking the DHEA rise seen after exercise.

Hypothesis

Global Hypothesis

That 50 mg of once daily administered DHEA replacement for 12 weeks in hypoadrenal women is associated with beneficial changes in insulin mediated glucose uptake, body composition, skeletal muscle protein metabolism, and mitochondrial function when compared to placebo.

Primary Hypothesis

That insulin mediated glucose uptake will be improved in hypoadrenal women after 12 weeks of DHEA replacement compared with placebo.

Primary Aim

To formally test insulin action using a hyperinsulinaemic euglycaemic clamp after 12 weeks of DHEA and after 12 weeks of placebo in a cross over study.

Secondary Hypothesis - 1

That after 12 weeks of DHEA replacement in hypoadrenal women there will be an improvement in skeletal muscle function – i.e. VO_2 max, dynamic and static strength.

Secondary Aim - 1

To measure changes in skeletal muscle function and exercise capacity using validated techniques before treatment and after 12 weeks of both placebo and DHEA.

Secondary Hypothesis - 2

That DHEA replacement will be associated with improvements in skeletal muscle protein metabolism and mitochondrial functions – specifically skeletal muscle oxidative / glycolytic enzyme oxidative ratios, and transcript levels of mitochondrial proteins.

Secondary Aim - 2

To measure skeletal muscle enzyme activity and mitochondrial function when on DHEA compared with placebo.

Methods

Subjects

The protocol was approved by the Institutional Review Board at Mayo Clinic, Rochester. The nature, purpose, and possible risks involved in the study were carefully explained to each subject before their informed consent was obtained. The recruitment process is summarised in Figure 7.

The target population was hypoadrenal women. The accessible population was women recruited from the Mayo Clinic surgical and medical indices, internet support groups for people with Addison's disease (www.healinglight.com, http://groups.yahoo.com/group/Addisons_Disease/), the National Adrenal Diseases Foundation newsletter and website (www.medhelp.org/nadf), the Cushing's Disease Support and Research Foundation (http://world.std.com/~csrf), and others. The intended sample and actual sample came from these sources (23 from Mayo held records and 10 from internet sources).

An initial search was made of the Mayo Clinic medical and surgical indices of hypoadrenal subjects seen at Mayo Clinic, Rochester, Minnesota in the 5 years prior to the start of enrolment into the study (i.e. May 1997 to May 2002). 1175 records were evaluated. Of these, 716 (60.9%) were women. 37 (3.1%) were women who had undergone total adrenalectomies with bilateral oopherectomies. Of the 716 women evaluated, 46 (6.4%) had died, 195 (27%) had either been seen only once, had no documentation available or lived greater than 500 miles from Mayo Clinic, Rochester (an initial recruitment consideration). A further 342 (47.7%) had exclusion criteria documented in the clinical notes.

A three-phase recruitment approach was used for the remaining 133 women. These women were initially invited by letter to take part in the study. There were 86 replies (64.7%). The non-responders were contacted again by letter 6 weeks after the first invitation. 3 replies (3.4%) were received in response to this second invitation. Of the 89 respondents, 52 expressed an interest in taking part in the study. The second phase of recruitment was that those subjects who expressed an interest in participating were contacted by telephone to have an initial exclusion criteria questionnaire. 23 of these 52 subjects (44%) had a previously undocumented exclusion criterion and were subsequently excluded from further participation. The final phase of recruitment was that the remaining 29 subjects were screened in the General Clinical Research Center at St Marys Hospital, Rochester. Of these 29 subjects, 2 subjects withdrew prior to the screening visit. 3 subjects failed the screen, 2 due to abnormal renal or liver function, and one due to a positive exercise stress test. One person passed the screen, but declined to take part in the study and was not randomised.

From the initial list of 1175, 23 (1.96%) subjects were randomised into the study. Of these, 1 person withdrew after 10 weeks in the first arm of the study due to protracted diarrhoea (on DHEA). The diarrhoea stopped on cessation of the drug. 2 subjects finished the first arm of the study, but declined to complete the second half (both on placebo).

It became clear that recruitment of the 30 necessary subjects would not be complete using the Mayo clinic indices. IRB approval was granted to allow an advertisement in the Minneapolis press, the National Adrenal Diseases Foundation newsletter, and also on the internet. The newspaper advertisement drew 2 responses, both of whom failed the telephone screen. The internet advertising was aimed at self help

groups for subjects with adrenal insufficiency. 185 replies were received from all over the world in the subsequent 6 months. Of these, a convenience sample was used. The first subjects who passed the telephone questionnaire were invited to be screened. These subjects came from all over North America. Distance from Mayo Clinic was no longer considered a limiting factor. Of the 11 subjects invited for screening, one failed due to abnormal thyroid function tests. 1 subject started the study and then withdrew prior to the 11 week visit (on DHEA). 1 subject unblinded herself by measuring her blood levels of DHEA during the first arm of the study (on DHEA).

Data is therefore presented of the 28 women who passed the screen and for whom complete data is available. All subjects were White and Caucasian. In addition to travel costs, subjects were compensated for their participation in the study.

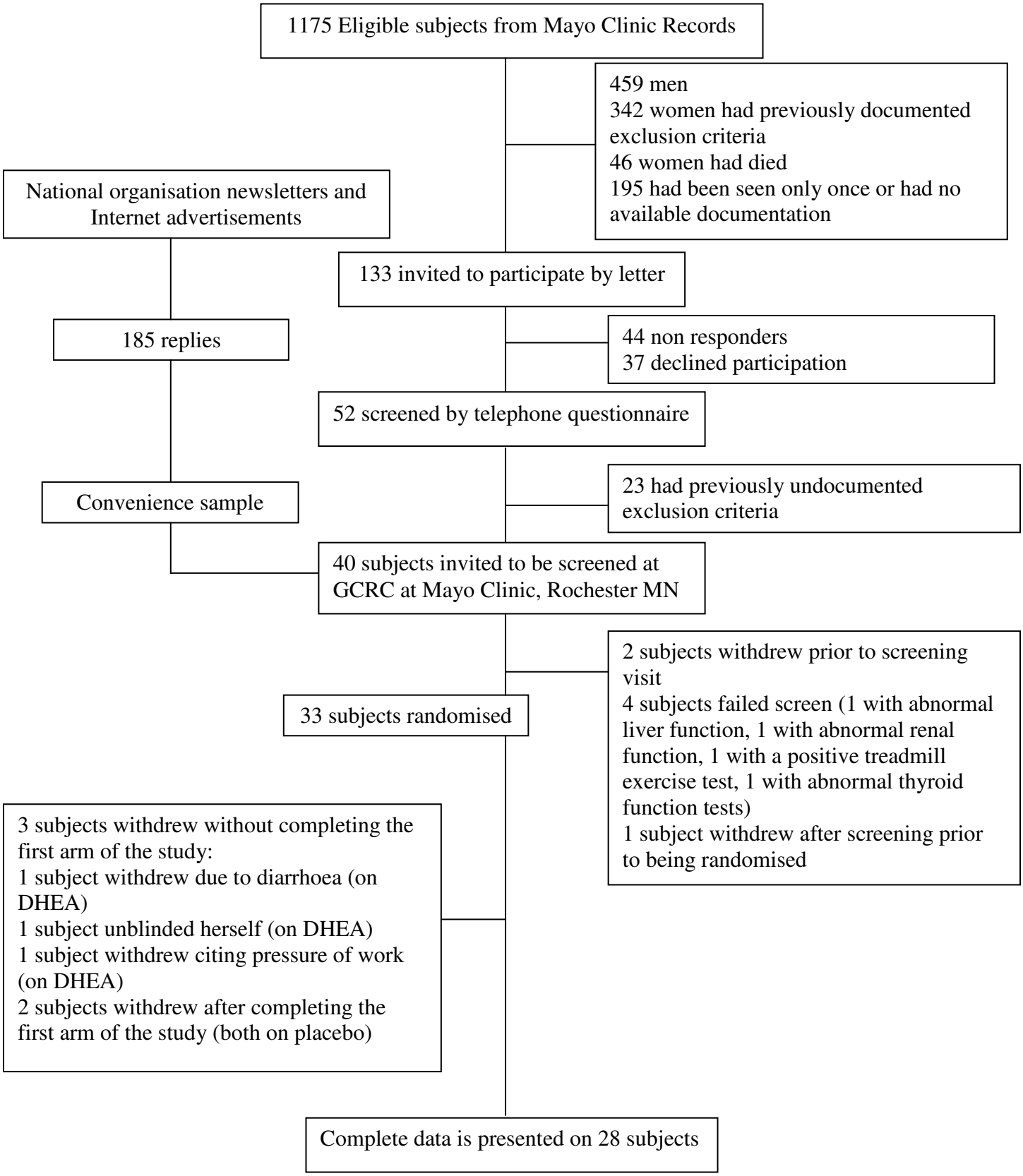


Figure 7 Recruitment Process

Inclusion and Exclusion Criteria

Inclusion criteria were a) subjects who had been adrenalectomised or had been hypoadrenal (from whatever cause) for greater than 24 months. b) Subjects who had been on a steady glucocorticoid replacement regime for greater than 12 months. c) Women of childbearing age in whom oestrogen status had been steady for greater than 6 months, i.e. either on or off the oral contraceptive pill for that time. d) Subjects on other forms of hormone replacement therapy (e.g. thyroxine) in whom the dose had remained the same for greater than 6 months.

Exclusion criteria were a) women with a BMI greater than 35 Kg/m^2. b) Those individuals with fasting blood glucose levels above 6.6 mmol/l (120 mg/dl). c) Those with a history of sex hormone dependant malignancy. d) Individuals with a history of liver disease or renal failure. e) A previous personal history of cardiovascular disease (other than hypertension) or polycythaemia. f) Women who were pregnant or breastfeeding. g) A history of cerebrovascular or neurological disorders. h) The use of drugs known to alter mood within the 6 months prior to enrolment or any drug known to affect hepatic biotransformation. Finally, i) postmenopausal women who had been on hormone replacement therapy for less than six months.

Drug Usage

The innovative aspects of the present study were the methods that were used. In addition to using the previously calibrated best dose of DHEA(S) – 50 mg per day, the subjects in the present study standardised the glucocorticoid replacement regime to try and decrease any confounding effects due to the multitude of replacement regimes available [171]. This had not been done in previous studies. Riedel et al evaluated the effects of three different cortisol replacement modes on subjective health status. These authors found that twice daily dosing was better than once daily, but that no regime normalised the health status to that seen in subjects with normal adrenal function [77]. The latter study involved a double blind cross over design involving 14 subjects and assessed wellbeing by the use of questionnaires

The mineralocorticoid replacement regime remained unchanged unless the screening urinary or serum electrolytes or blood pressure suggested that the regime that the subject was on at the start of the study was inappropriate. There is evidence that mineralocorticoid antagonist therapy (i.e. spironolactone - there is currently no data for eplereonone) decreases DHEAS levels in both sexes [172;173]. Spironolactone therapy also increases serum cortisol, so raising the cortisol/DHEA(S) ratio [174]. However, there is no evidence that adequate mineralocorticoid replacement therapy (i.e. fludrocortisone) has a detrimental effect on physiological function.

Standardisation onto hydrocortisone 10 mg on rising, 10 mg at 4 pm and 5 mg at bedtime was attempted from 3 weeks prior to entry into the study. If subjects were on either a lower equivalent dose of prednisone or a lower overall hydrocortisone dose prior to entry into the study then they were asked to stay on their current dose. For those on

hydrocortisone, subjects were asked to go to at least a twice or three times a day regime. All of these changes were made with full approval of the volunteer's primary physician and/ or endocrinologist. Average total daily dose at entry into the study was 24.4 ± 7.0 mg, divided between 1 and 3 times per day. 8 subjects had been on prednisone (average dose 4.5 ± 1.4 mg), and been changed to hydrocortisone several weeks prior to randomisation.

Two subjects had previously been on commercially available DHEA. One had been on 25 mg per day and the other had been on 50 mg per day. Both subjects stopped the DHEA at least 6 months prior to starting the study.

The dose of 50 mg of DHEA has been shown to be the appropriate dose by assessing the 24 hour urinary excretion of androstenedione glucuronide, an androgen metabolite regarded as a reliable marker of the pool of testosterone [34]. This increased to levels seen in normal young women in accordance with the report from Arlt et al [21], who noted serum levels of this metabolite in the upper normal range 24 hours after the intake of 50 mg of DHEA.

Study Protocol

This was a single daily dose, randomised, placebo controlled, crossover design performed at a single centre. The study was designed to look at the effect of 50 mg of DHEA given daily for 12 weeks on body composition, muscle strength, insulin sensitivity and skeletal muscle physiology in 33 hypoadrenal women, compared to placebo. The study took place from May 2002 to July 2003. Each subject was in the study for a minimum of 26 weeks. Time between screening and entry into the study varied from 1 day to 3 weeks, and time between both arms of the study varied between 2 weeks and 4 months.

There were potentially a total of 7 visits to the General Clinical Research Centre during the study. Six of these visits are shown in Figure 8.

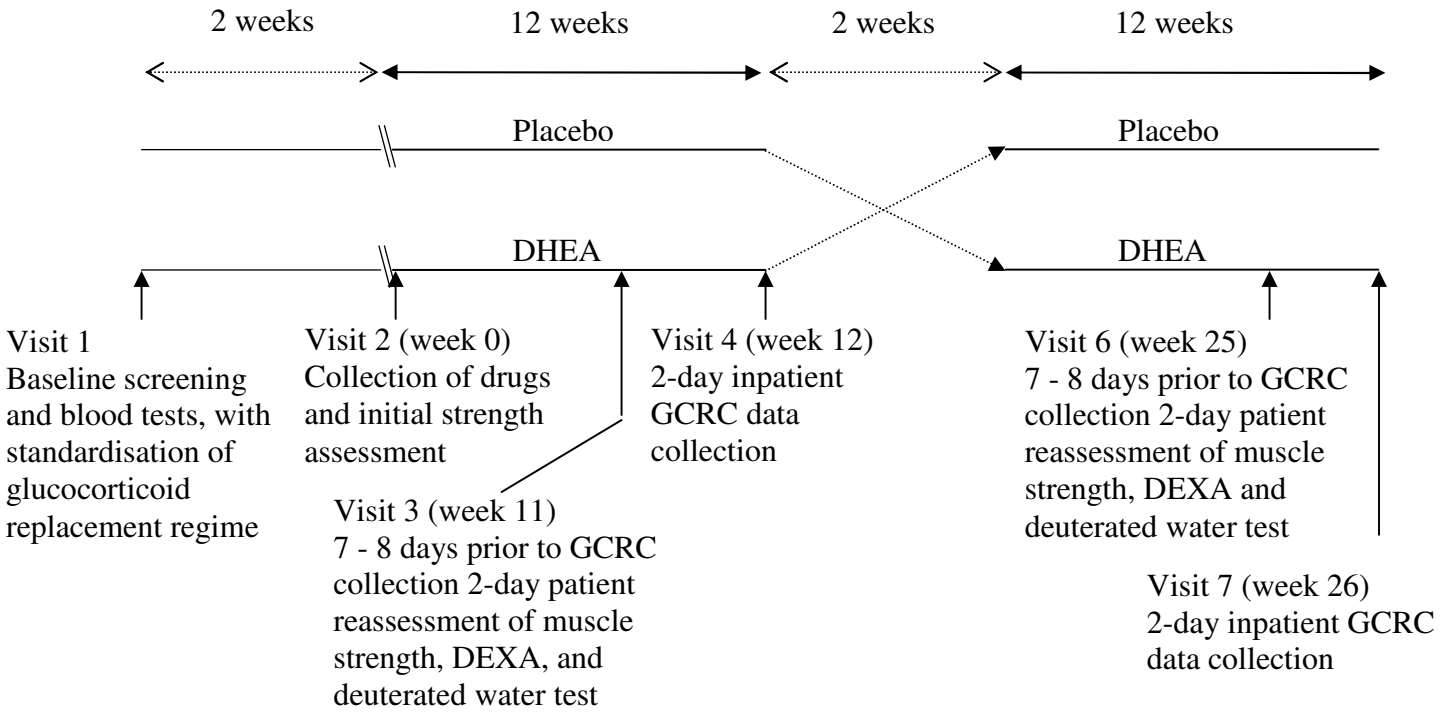


Figure 8 Overall Study Outline

Visit 1 (Initial screening visit)

During this visit subjects had the study explained to them in detail and informed written and verbal consent obtained. A physical examination was done, including measurement of total body mass to 0.1 Kg, and height, measured in centimetres. Body mass index was calculated from these measurements (Kg/m^2). Blood was taken to assess baseline values of a renal and liver function, as well as plasma glucose and a full blood count. DHEAS levels were also checked to ensure low levels.

Subjects underwent a standard, submaximal treadmill exercise stress test to verify the absence of any cardiorespiratory abnormalities. During the test, continuous monitoring of 12-lead electrocardiogram, blood pressure, and expired gases were performed. All subjects were encouraged to give maximal performance during the test as assessed by the Borg index of relative perceived exertion [175]. The test also increased familiarity with the testing environment, equipment and procedures. The exercise test was to evaluate cardiovascular reserve and allow calibration of the stationary bicycle. Briefly, after 2 minutes of walking at a previously determined speed (0% grade), the grade was increased 2.5% every two minutes until the subject was unable to continue. Based on previous use of this protocol it was anticipated that fatigue would be reached within 7 to 12 minutes, excluding the warm-up period.

The results of the tests were then evaluated and the subjects contacted that afternoon to let them know if they had passed the screen or not. If they had, they were asked if they wanted to continue in the study after having a chance to read the consent form and consider all of the written and verbal information they had been given during

the screening visit. If they chose to participate, a schedule was arranged for the next three visits. These were designated week 0 (Visit 2), week 11 (Visit 3), and week 12 (Visit 4).

Visit 2 (Also known as week 0).

At this visit the volunteers returned to the GCRC to have the initial set of psychological assessments under the supervision of a trained psychometrist. This was done to assess the psychological effects of DHEA in these subjects. That data is presented elsewhere [79]. Subjects also collected the study drug. Pharmaceutical grade DHEA (Gardena, CA) was given at a dose of 50 mg per day or placebo and taken at the same time every day. Both placebo and DHEA were identically encapsulated (Clinical Encapsulation Services, Schenectady, NY). Pregnancy status was assessed in premenopausal women and during the course of the study these volunteers were advised against getting pregnant. However, no measures were taken to ensure the premenopausal subjects were in the same stage of the menstrual cycle when blood samples were drawn.

Strength tests were also done at this visit. These are described later in this section.

Visit 3 (Also known as week 11)

By the time of this visit the subjects had been taking the drug for 11 weeks. The details of this visit are shown in Figure 9. At this 2-day visit, the subjects had a haemoglobin level measured to ensure that they were not anaemic prior to the muscle biopsies due to take place one week later. Subjects then underwent repeat psychometric testing, and VO_2 max was measured by the use of a stationary bicycle on the morning of the first day. A DEXA scan was carried out that morning. They had repeat strength

testing in the afternoon of the first day. On the morning of the second day the subjects had their total body water measured using deuterated water to measure body composition. Subjects also had indirect calorimetry measured to assess resting energy expenditure. These are described in the next section.

The strength tests were done at this visit (1 week before visit 4 at 12 weeks) as this ensured that there was no damage to the skeletal muscle fibers or changes in enzyme activity prior to biopsy that could have confounded the results. Subjects were asked not to do any vigorous exercise until after the next visit. They were also asked not to take any non-steroidal anti-inflammatory drugs (including aspirin) until after the next visit.

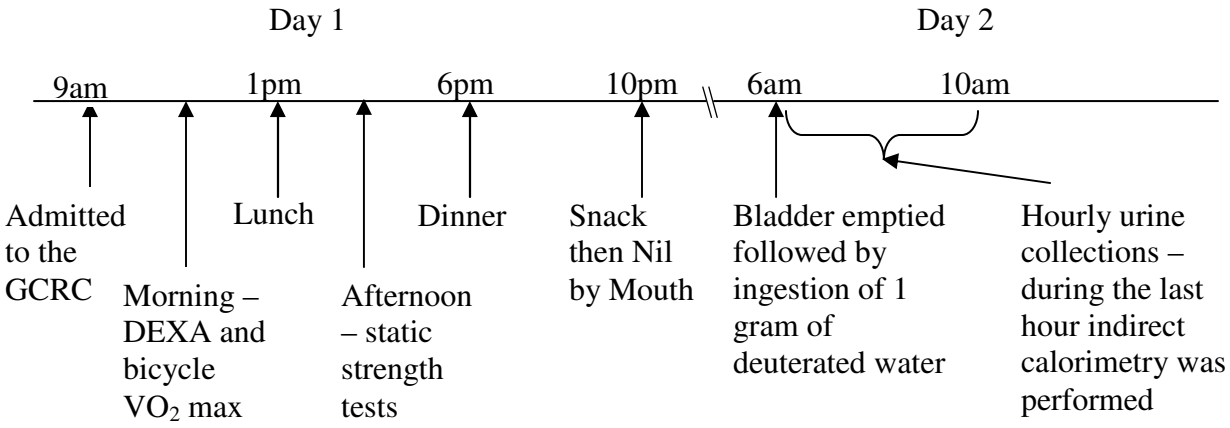


Figure 9 Details of GCRC Collection – 1st stay (week 11 and week 25)

GCRC – General Clinical Research Centre, DEXA – Dual Energy X-Ray Absorbtiometry

Visit 4 (Also known as week 12)

This was another 2 day visit. The details of this visit are shown in Figure 10. At this visit subjects were admitted to the GCRC at 17:00 h. All subjects were on a standard weight-maintaining diet. The nutritional values of these meals were divided as follows:

carbohydrate 55%, protein 15%, fat 30%. These meals were provided either from the GCRC for 3 consecutive days prior to each inpatient study period, or prepared by the subjects from an individualised detailed dietary advice sheet given to them by the research dieticians. These instructions were provided to those subjects who lived some distance from the GCRC for whom daily provision of meals was not possible. Subjects had the same dietary pattern before both the 12 and 26-week visits.

One subject was on warfarin. Her INR was checked the day of admission for her biopsies. On both occasions her INR was less than 2.5, and as a precaution, the primary investigator performed her biopsies. Extra precautions were taken to ensure adequate haemostasis had been achieved at both biopsy sites prior to discharge, with emergency contact details given in case of necessity.

On the second day all subjects underwent hyperinsulinaemic euglycaemic clamping for measurement of insulin sensitivity, and skeletal muscle needle biopsy for measurement of levels of mixed muscle protein, mitochondrial protein, and sarcoplasmic protein. In 7 subjects, synthesis rates of these proteins were also measured. This necessitated infusions of labelled amino acids. This is shown in Figure 11. The muscle samples were also used to measure levels of mitochondrial enzyme activity. These are described in detail in the next section.

It can be seen from Figure 10 that 6, 6 $^{2}H_2$ glucose (D_2 glucose) was infused from 4 am to 7 am. This was used to assess fasting glucose turnover.

It was just prior to the clamp starting and during the clamp that blood was taken to measure levels of hormones, including insulin, glucagon, and cortisol. Fasting lipids, insulin like growth factors and their binding proteins levels were also measured.

The subjects were then taken off the study drugs for a minimum of 2 weeks. This length of time was chosen as it had previously been described that the half-life of DHEAS in women was 20.8 hours [35]. We assumed that the time taken for complete elimination was 5 half-lives, thus 2 weeks was more than sufficient to ensure that there would be no carry over effects of DHEA. In addition, as no measurements were being taken at the end of the 2 weeks, indeed, no measurements of any kind were being taken at least 14 weeks after the first set of biopsies; it was assumed that 2 weeks wash out would be sufficient.

Visit 5 (First visit after the washout period)

This visit, at the start of the second arm of the study is not shown in Figure 8, as this only involved picking up the drugs for the second arm of the study. If subjects lived more than 100 miles from Mayo Clinic, Rochester, then this visit was omitted and the drugs either sent to the volunteers by post, or the drugs were dispensed at the week 12 visit when subjects came to the end of the first arm of the study to save them a journey.

As mentioned, this visit varied between 2 weeks and 4 months after the end of the first phase of the study depending on subject preference and availability.

Visit 6 (Also known as week 25)

This visit was 11 weeks into the second phase of the study, 25 weeks after starting the study (if there was a minimum of the two week break between the 2 halves of the study). This visit was identical to visit 3 (week 11) and is illustrated in Figure 9.

Visit 7 (Also known as week 26)

This was the final visit, at week 12 of the second arm of the study. It involved the muscle biopsy and hyperinsulinaemic clamp. This visit was identical to visit 4 and is illustrated in Figures 10 and 11.

Subjects were contacted every two weeks during the study by telephone or email to ensure compliance and also to assess any adverse effects of the study drug.

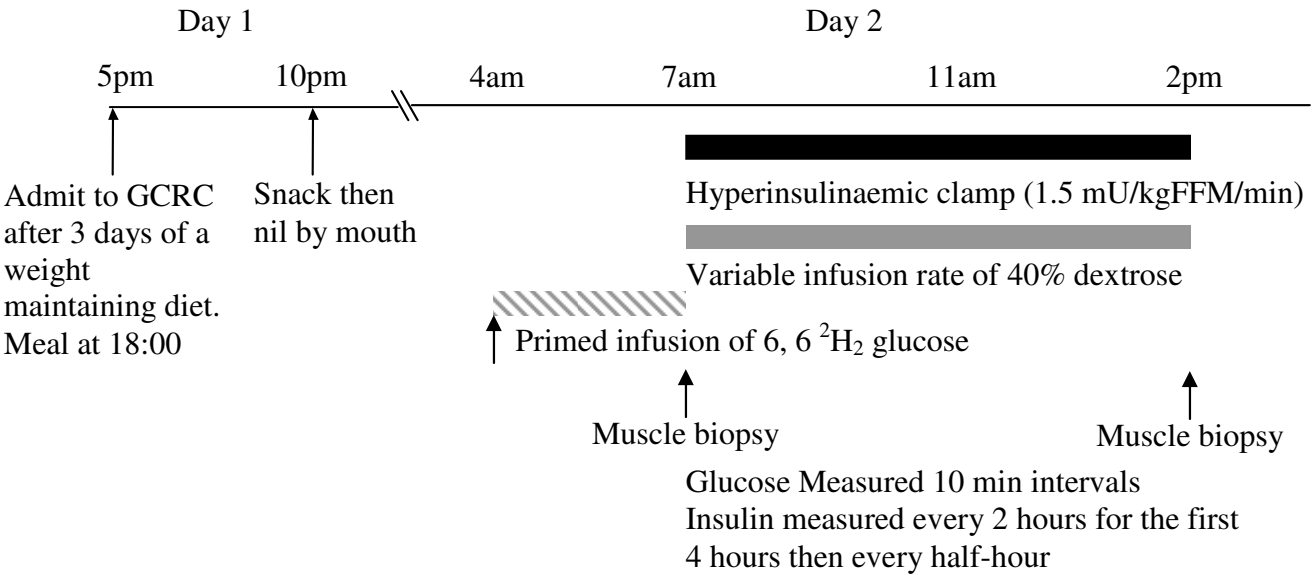


Figure 10 GCRC Visit Week 12 and 26

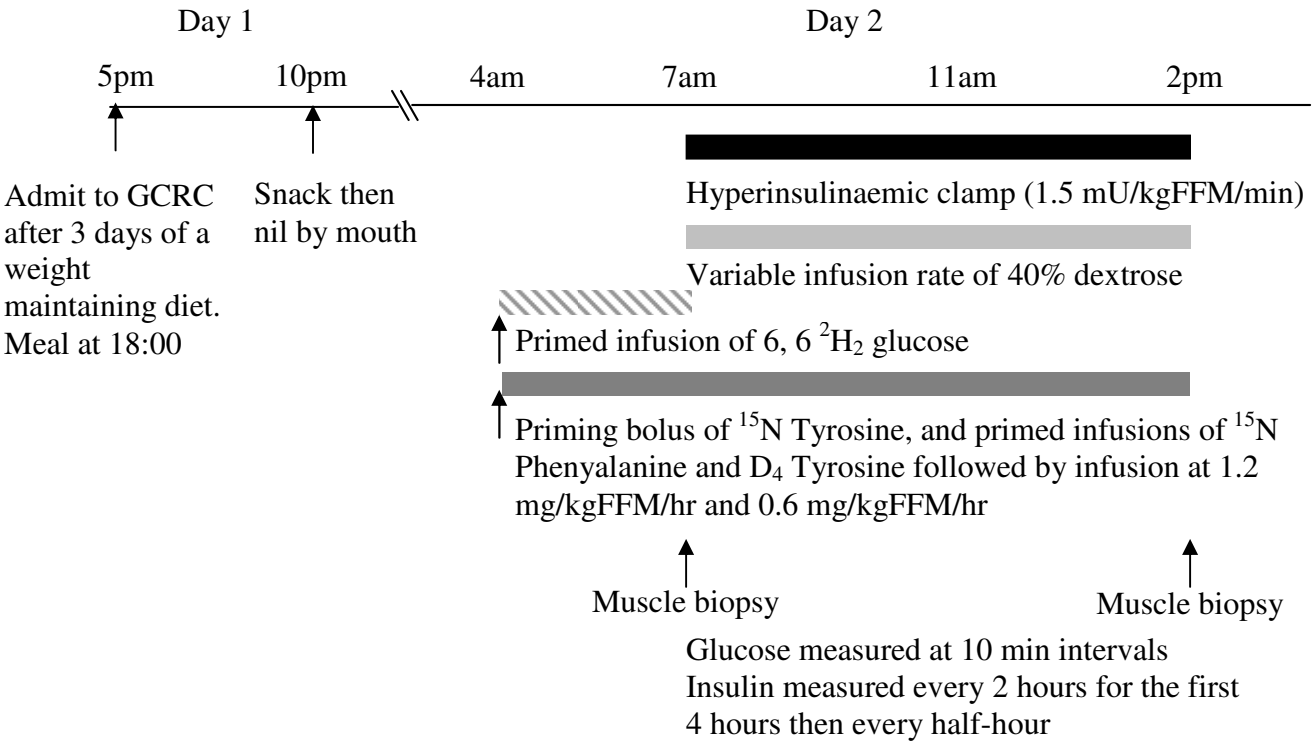


Figure 11 GCRC Visit Week 12 and 26, with Amino Acid Infusions

Experimental Methodology

Strength Tests

The volunteers were subjected to strength tests of the upper and lower body. These were performed in the Dan Abraham Healthy Living Center exercise facility. The subjects were familiarised with the strength equipment to reduce test anxiety. A one-repetition maximum (1RM) was established on the seated chest press, bicep curl, knee extension, and leg curl machines in all subjects. One subject was unable to lie down for the biceps curl and is 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 analysed in absolute figures, but no comparison was possible with baseline.

The 1RM is the highest weight that can be lifted one time. In each case, the subject performed a series of 1 to 3 repetitions, each at progressively higher resistance loads, until the 1RM was established.

Isotonic strength testing was performed on a Universal weight. All strength testing was conducted by the principle investigator or by the study co-ordinator. Experienced, American College of Sports Medicine-certified personnel had trained both the principle investigator and the study co-ordinator.

Warm-up consisted of mild static stretching and several repetitions of the exercise using a lightweight. A starting weight was set at approximately 70% and 100% of each subject's body weight for the bench press and double leg press, respectively. After each successful repetition, the weight was increased until the subject could not lift the weight through their full range of motion despite verbal encouragement. To minimise muscle fatigue, approximately 2 minutes rest was given between trials and attempts were made to

determine the 1RM for each strength test within 6 trials. Three to four minutes of rest were allowed between the bench press and double leg press tests. Subjects who did not take routine exercise were warned that they would ache for the next 48 hours or so, and to take acetaminophen for the pain.

Subjects also performed a static strength test, by assessing peak handgrip, and thus forearm flexor strength. This was done in the GCRC Exercise Core Lab. The subjects were seated with the shoulder adducted and neutrally rotated, the elbow flexed at approximately 20 degrees and the forearm in a neutral position.

DEXA

Fat mass and fat free mass (in kilograms) were determined using a full size dual energy X-ray absorptiometry (DEXA) scan (Lunar, Madison, WI). The subjects were asked to remove all metal objects and lie flat in a supine position on the DEXA table. The speed of the scan was determined by the thickness of the subject's chest.

For DEXA data interpretation, body weight was initially considered to consist of two components: total body bone mineral and total body soft tissue mass. These two components were determined by measuring the attenuation of two x-ray energies. The soft tissue mass was then partitioned into fat mass and fat free mass. The proportion of each proponent was calculated from the ratio of mass attenuation coefficients of the two energies - the ratio of soft tissue absorption. Corrections were made for tissue thickness and composition. The scanner was routinely calibrated by scanning plastic calibration phantoms that contained bone and soft tissue components. In addition, meat blocks of known fat content composing a range (5.3% to 70.2% fat) of known quantities of fat and lean tissue (Hormel Foods Corp, Austin, MN) were scanned on a monthly basis. For bone mineral density (BMD) quality control was addressed in two ways:

a) Acceptance testing - this involved examination of the effects of patient variables such as body thickness on the reproducibility of BMD measurements. This was done using a simulated spine phantom and acrylic blocks to simulate varying body dimensions.

b) Routine quality control - this involved daily scanning of a spine phantom and weekly scanning of an acrylic block that simulated soft tissue.

Bicycle VO_2 Max Testing

On the morning of either day 1 or day 2 of the 11 and 25-week visits, the subjects had their VO_2 max measured using an incremental protocol on an electrically braked bicycle ergometer. After an initial 3-minute stage at 15 to 50 Watts, workload was increased by 5 to 25 Watts every minute until volitional fatigue was achieved. Expired gases were measured were analysed using Perkin Elmer spectrometer and MedGraphics software [176]. The initial settings on the bicycle were determined by the results of the VO_2 max done at screening.

Oxygen consumption and carbon dioxide production were measured using the open-circuit indirect calorimeter system on a SensorMedics 2900 metabolic measurement cart (SensorMedics, Yorba Linda, CA), at rest, during exercise and during recovery from exercise. An individual's VO_2 max was calculated by averaging the highest three values of oxygen consumption during the test. To ensure that VO_2 max had been achieved, the following criteria had to be met: a) the subject's heart rate had to be close to the maximum predicted heart rate, (220 minus subject's age), b) the perceived rate of exertion had to exceed 17 [175], c) a plateau VO_2 was observed with increasing workload, and d) the respiratory exchange ratio at peak intensity exceeded 1.1.

Total Body Water

Subjects had an evening meal at 18:00 h followed by a snack at 22:00 h. They then remained nil by mouth until 06:00 h when they voided their bladder and drank 1 gram of deuterated water, followed by 4 urine collections at one hourly intervals. As there were no differences seen in body composition using DEXA, it was felt that the

additional information that total body water may yield was minimal, and thus this urine has not yet been analysed.

Indirect Calorimetry

During the last hour of the urine collection, subjects lay quietly in the supine position in a darkened, thermoneutral room to enable measurement of resting energy expenditure by indirect calorimetry. Subjects were supplied with earplugs to reduce auditory stimulation. Subjects were also asked to refrain from moving their limbs during the assessment. Oxygen consumption and CO_2 production were measured using the DeltaTrac Metabolic Cart using the ventilated hood technique. The metabolic rate is thought to be the lowest, waking, energy expenditure. Energy expenditure can be measured indirectly with a metabolic cart by analysis of expired gases to derive volume of air passing through the lungs, the amount of oxygen extracted from it (i.e., oxygen uptake - VO_2), and the amount of expelled carbon dioxide (CO2 output – VCO_2) – all computed to represent values corresponding to 1 minute time intervals.

Carbon dioxide and oxygen volumes were averaged, converted to kilocalories using the Weir equation - (VCO_2 x 1.106) + (VO_2 x 3.941) [177], and expressed on a per minute basis by dividing the result by 1440. RQ was calculated as VCO_2/VO_2. Fat and carbohydrate oxidation rates were estimated by calculations based on the average respiratory exchange ratio using the caloric equivalents of Livesey and Elia [178].

Following the urine collections and indirect calorimetry, subjects were discharged. Due to scheduling difficulties in the GCRC, a small number of subjects had to have their bicycle VO_2 max done on the morning of day 2.

Hyperinsulinaemic Euglycaemic Clamps and Fasting Glucose Turnover

After admission at 17.00 on weeks 12 and 26 (visits 4 and 7), subjects ingested a standard meal at 18:00 h and snack at 22:00 h. Thereafter the subjects remained in a fasting state until the end of the inpatient period the following day. One retrograde and one antegrade intravenous cannulae were placed into veins on the back of the non-dominant hand.

At 06:00 h the subjects were awoken and they remained awake for the rest of the study. Arterialisation of superficial venous blood was achieved by placing the non-dominant hand in a hot box at 120^{o}F from 06:00 h for the duration of the study [179]. Subjects remained supine for the rest of the study. At 04:00 h of each inpatient period a priming dose of 40% 6, 6 $^{2}H_2$ glucose (D_2 glucose) at 3 mg/KgFFM was injected through a cannula in the back of the contralateral hand to assess fasting glucose turnover. This was started while the subjects were asleep. An infusion of $^{2}H_2$ glucose was then continued at 3 mg/KgFFM/hr until 07:00 h. At 0:700 h the $^{2}H_2$ glucose infusion was stopped. Insulin (1.5 mU/kgFFM /min) was begun at 07:00 h. Arterialised blood glucose was measured every 10 min with a Beckman Glucose Analyser (Beckman Instruments, Fullerton, CA). To maintain euglycaemia during high physiological insulin, the rate of glucose (40% solution) infusion was adjusted accordingly. This was done to measure insulin sensitivity. The study outline is illustrated in Figure 10.

Muscle Protein Synthesis

During the course of the study it was decided that 8 subjects were to be studied with additional infusions of [^{15}N]phenylalanine (^{15}N phenylalanine) and [ring 2,3,5,6-$^{2}H_4$]tyrosine (D_4 tyrosine) to establish fractional synthesis rates of mixed muscle protein, mitochondrial proteins and sarcoplasmic protein subfractions. One of these 8 subjects unblinded herself during the first arm of the study. Complete data from the remaining 7 subjects are shown for comparative data between placebo and DHEA. The study outline for these 7 subjects is shown in Figure 11.

In the subjects in whom amino acids were infused, priming doses of ^{15}N phenylalanine at 1.6 mg/KgFFM, ^{15}N tyrosine at 0.6 mg/KgFFM and D_4 tyrosine at 0.6 mg/KgFFM were given at 04:00. Infusions of ^{15}N phenylalanine and D_4 tyrosine were then continued at 1.6 mg/KgFFM/hr and 0.6 mg/KgFFM/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 infusions.

Muscle Biopsies

These were done at visit 4 (week 12) and visit 7 (week 26). The first biopsy was done prior to the start of the insulin infusion at 07:00 for the hyperinsulinaemic clamp. The second biopsy was taken from the contralateral thigh after 7 hours, at 14:00. As mentioned, subjects were instructed not to exercise for 48 hours prior to biopsy or to take non steroidal anti-inflammatory drugs. Vastus lateralis muscle samples (~300 mg each) were obtained under local anaesthesia (lidocaine, 2%), with a percutaneous needle as previously described [180-182].

Muscle for protein synthesis measurements and enzyme activities was immediately frozen in liquid nitrogen and kept at $-80^{o}C$ until analysis.

Safety Monitoring

As part of the ongoing assessment within the study, all of the blood tests were looked at by one of the principle investigators within 48 hours of the results being made available. Rules about aberrant results had been discussed a priori. These were to have been to determine if an aberrant result constituted an 'adverse event'. If an adverse event were to be noted then the subject was to be taken off the drug and given the opportunity to be followed on an 'intent to treat' basis. This did not occur on any subjects, although one subject experienced diarrhoea during the first arm of the study whilst on DHEA. She stopped taking part in the study after 10 weeks. She declined to take any further part in the study. Subjects were to be asked to perform the strength tests and questionnaires. If they were on the first arm of the study they would be offered the chance to go onto the second arm. In addition, a physician was present during exercise stress testing and VO_2 max tests.

Metabolite Concentrations

Plasma levels of amino acids were measured by a high-performance liquid chromatography system (HP 1090, 1046 fluorescence detector and cooling system) with t-butyldimethylsilyl ether derivatisation under electron ionization conditions [183]. Glucose was analysed on site by an analyser using an enzymatic technique (Beckman Instruments Inc., Fullerton, CA).

Hormonal Assays

DHEAS was measured using a competitive RIA from Diagnostic Systems Laboratories (Webster, TX). The intra-assay coefficient of variations were 6.4% at 0.74 μg/mL and 6.9% at 2.83 μg/mL (3.7% at 220 ng/ml and 1.9% at 2000 ng/ml) [184]. The lowest level of detection was 0.3 μg/ml.

Insulin was measured using the Access® Ultrasensitive Insulin Assay (Sanofi Diagnostics Pasteur, Chaska, MN). This was a fully automated procedure using two monoclonal antibodies with a chemiluminescent detection system. The assay had negligible cross reactivity with proinsulin and C-peptide. The precision varied from 5.6% at 0.15 μIU/ml, to 4.0% at 0.30 μIU/ml, to 3.1% at 100 μIU/ml. The minimum detection limit was 0.03 μIU/ml.

Free IGF 1 was measured using a two-site immunoradiometric assay from Diagnostic Systems Laboratories (Webster, TX) [185]. The assay had a minimum detection of 0.03 ng/ml and had interassay precision of 14% at 1.9 ng/ml and 10% at 5.1 ng/ml. No cross-reactivity was noted for IGF 2, insulin, proinsulin, or growth hormone [186]. The free

IGF 1 was separated from the bound at the end of the assay by using a coated tube. The separation of IGF 1 from the binding protein prior to assay to measure total IGF 1 used an ethanolic hydrochloric acid solution to liberate the IGF 1 followed by centrifugation and neutralisation of the supernatant which was then assayed.

Total IGF 1 was measured with a two-site immunoradiometric assay from Diagnostic Systems Laboratories (Webster, TX). The assay had a minimum detection of 0.8 ng/ml and precision of 9% at 64 ng/ml and 6.2% at 157 ng/ml. No cross reactivity was noted for IGF 2, insulin, proinsulin, and growth hormone [187].

IGF BP1 was measured using a two-site immunoradiometric assay from Diagnostic Systems Laboratories (Webster, TX). The assay had an intra-assay CV of 6.5% at 0.5 ng/ml. No detectable cross-reactivity was seen with binding proteins 2 to 6. The minimum detection level was 0.5 ng/ml [188].

IGF BP3 was measured using an immunoradiometric assay from Diagnostic Systems Laboratories (Webster, TX). The assay had an inter-assay CV of 1.0 to 1.9%. The minimal detection dose was 2.0 ng/ml [185].

Sex-Hormone Binding Globulin was measured with a two-site immunoradiometric assay from Diagnostic Systems Laboratories (Webster, TX). The intra-assay CV is 5.0% at 48 nmol/L and 5.5% at 117 nmol/L. The lowest detectable level was 5 nmol/L [189].

Testosterone was measured with the coat-a-count RIA (DPC, Los Angeles, CA). The assay had 20% cross-reactivity with nontestosterone, 3.3% with dehydrotestosterone, and 1.7% with methyltestosterone. The inter-assay CV was 11% at 76 ng/dL, 6.4% at 264 ng/dL, and 6.0% at 672 ng/dL. The lowest measurable level was 4 ng/dL.

Bioavailable testosterone was measured by the concentration of H3 testosterone not precipitated by ammonium sulphate in the sample was divided by the concentration of H3 testosterone not precipitated by ammonium sulphate in a 5% bovine serum albumin sample containing no sex hormone binding globulin.

Cortisol was measured on a Beckman Access instrument, using a Beckman kit (Beckman Coulter Inc, Fullerton, CA). The lower limit of detection for this assay was 0.4 ug/dL, with an interassay CV of 9.4% at 3.25 ug/dL, 5.8% at 21.3 ug/dL, and 5.1% at 35.5 ug/dL.

Androstenedione was measured by direct radioimmunoassay, from Diagnostic Systems Laboratories (Webster, TX). The lower limit of detection for this assay was 0.1 ng/mL, with an interassay CV of 4.1% at 0.92 ng/mL, and 4.2% at 5.10 ng/mL.

Skeletal Muscle Analysis

Mixed Muscle Protein Synthesis

Briefly, this technique involved initially pulverising a 50 mg muscle sample at liquid nitrogen temperature. Protein in the tissue was precipitated by the addition of 6% perchloric acid. The mixture was spun for 30 minutes at 3000 RPM. The supernatant was then separated and kept for measuring tissue fluid isotopic enrichment. The precipitated protein was washed in petroleum ether and then dissolved in 0.8 M sodium hydroxide at 60°C and insoluble residue was removed by centrifugation at 3000 RPM for 30 minutes. The supernatant was then hydrolysed with 6 M hydrochloric acid at 110° C for 18h. The hydrolysate was dried in a speed vacuum. A 150 mg portion of each muscle was used for the isolation of mitochondrial and sarcoplasmic protein fractions by differential centrifugation as previously described [104;106;190].

Isolation of Mitochondria and Sarcoplasmic Protein from Muscle Samples

After homogenising the muscle in a sucrose, EDTA and Tris buffer, mitochondrial and sarcoplasmic protein were separated from a fraction containing the myofibrillar and nuclear protein and the connective tissue by centrifugation at a low speed (600 x g). The mitochondrial protein was separated from the sarcoplasmic protein by centrifugation at a higher speed (7000 x g). The pellet containing the mitochondrial protein was washed using a potassium chloride, magnesium sulphate, ethylene glycolbisaminoethylether tetracetic acid, ATP and Tris buffer. The purity of the mitochondrial fraction was tested by assessing the contamination with other membrane structures [191]. The sarcoplasmic protein fraction was purified from mechanistic structures by spinning at 100,000 x g.

Skeletal muscle contains two mitochondrial subpopulations: subsarcolemal and intermyofibrillar mitochondria. This latter population is tightly bound to the myofibrils and can only be separated by using a proteolytic enzyme to free them from the myofibrillar proteins. Although different characteristics for both subpopulations have been described, these could well be the results of the proteolytic enzyme used to obtain the intermyofibrillar mitochondria [192]. Incubation with the proteolytic enzyme, however, interferes with the measurements of MHC synthesis rates. Therefore, only synthesis rates of the subsarcolemal mitochondria were studied.

Mitochondrial Enzyme Activities

Aliquots of muscle homogenate from the second biopsy were used to measure the activities of citrate synthase and cytochrome c oxidase, two representative mitochondrial enzymes [104;193] to obtain an estimation of both muscle oxidative capacity from the quantity and the quality of the mitochondria. Activities of citrate synthase and cytochrome c oxidase were measured because of the importance of these enzymes in the flux-generating steps of the tricarboxylic acid cycle, and the electron transport chain respectively.

A separate piece of muscle was used to isolate free tissue fluid as previously described [194]. Briefly, this involved a sample of tissue being homogenised in 1M perchloric acid. Samples were then centrifuged at 3000 x g for 30 minutes.

Enzyme activities are measured with a spectrophotometer that measures the density of light passing through the reaction tube.

The samples were run on a single cuvette machine. Oxaloacetate and acetyl CoA were added, with the 2 components being condensed. To follow the reaction, dithionitrobenzoate was added. This compound has a sulphydryl group, and accepts the CoA moity. Dithionitrobenzoate has peak light absorbance at 412nm. As the reaction proceeded the solution became progressively darker yellow, which caused the absorbance reading on the spectrometer to increase. This reaction was linear for several minutes.

Because citrate synthase is a stable enzyme the reaction was allowed to occur at room temperature.

No standard curves for enzyme activity measures were used as it was a kinetic measurement. The main criterion was that the slope was linear over the 1-3 minutes that

the reaction was followed. The appropriate amount of sample required to keep it linear was determined by trial and error when the assay was set up. The reagent cocktail was mixed in a cuvette, an aliquot of sample was added, and the absorbance was recorded at regular intervals on the spectrophotometer. The slope of absorbance over time was used to calculate the rate. Cytochrome c oxidase is the enzyme that uses oxygen in mitochondria (complex IV). Activity was measured by adding reduced cytochrome c, which had high absorbance at 550 nm (i.e. very red), and then followed the oxidation by the linear decline in absorbance (by turning pink). Commercially available purified horse heart cytochrome c was used (Sigma-Aldrich, St Louis, MO). The iron group was reduced with ascorbic acid before use.

Mass Spectroscopic Quantification of Muscle Amino Acid Levels

The supernatant containing the free pool of amino acids was removed and purified by means of a cation exchange column (AG 50W x 8 resin, 100 – 200 mesh, hydrogen form; Bio-Rad Laboratories, Richmond, CA). Acidifed samples were applied to the column, and amino acids were eluted with 2 ml of 4N NH_4OH. The NH_4OH fraction was evaporated to dryness in a Speed Vac for subsequent analysis by mass spectroscopy. The samples required derivatisation as their t-butyldimethylsilyl ether for analysis of isotope enrichment by mass spectroscopy.

Mass Spectroscopic Analysis

For measurement of the isotope enrichments a suitable ion fragment containing the natural and isotope labelled species was chosen for the mass spectrometer to monitor. In the case of glucose, fragments having masses at m/z (mass to charge ratio) 319 and 321 were chosen which contain the natural form, and the dideuterated atoms respectively. The mass spectrometer produced 2 traces, one each for m/z 319 and 321, and the area under the glucose peak for each of these was measured. The area ratio of 321/319 gave a value that was compared to a calibration curve of known glucose enrichments to obtain the enrichment value of the unknown.

The muscle protein fractions were hydrolysed overnight in 0.6 mol/l HCl in the presence of cation exchange resin (AG-50; BioRad, Richmond, CA) and purified the next day using a column of the same resin. The amino acids were dried (SpeedVac; Savant Instruments) and then derivatised as their t-butyldimethylsilyl ether. ^{15}N phenylalanine enrichments in muscle proteins were determined using a gas chromatograph-combustion isotope ratio mass spectrometer (GC-c-IRMS; Finigan MAT, Bremen, Germany) as described [108]. Tissue fluid amino acids were derivatised as their t-butyldimethylsilyl ester derivatives and analysed for ^{15}N phenylalanine enrichments using a gas chromatograph-mass spectrometer (GC-MS; Hewlett-Packard Engine, Avondale, CA) under electron ionisation conditions [108].

Calculations

Glucose

Glucose appearance (Ra) and disappearance (R_d) (or glucose turnover) was calculated using the steady state equation:

$R_d = R_a$ = [Infusion rate of $^2H_2$ glucose / plasma enrichment of $^2H_2$ glucose] – infusion rate of $^2H_2$ glucose.

Protein

The fractional synthesis rate (FSR) of mixed muscle protein, mitochondrial and sarcoplasmic proteins was calculated using the equation [195]:

$$FSR\ (\%/h) = 100 \times (E_{10h} - E_{3h})/(E_{TF} \times t)$$

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, which in this case was 7 hours (mean 6.9 hours, 95% CI 6.82, 7.04). The synthesis rates were calculated using ^{15}N Phenylalanine, ^{15}N Tyrosine and D_4 Tyrosine as the precursor amino acid. The results did not differ regardless of what precursor amino acid was used. The data using ^{15}N Tyrosine and D_4 Tyrosine is not shown, however, the data that is presented used ^{15}N Phenylalanine as the precursor.

Calculation of Insulin Sensitivity

Insulin mediated glucose uptake was calculated as milligrams of glucose infused per minute per kilogram of fat free mass during the hyperinsulinaemic euglycaemic clamp. This was expressed as an M value.

Statistical Analysis

Statistical analyses were done using Wilcoxon sign rank tests. Analysis was done to assess changes of endpoints from pre-treatment to post-treatment within each treatment as well as to compare these changes between treatments. An analysis was only performed on those subjects for whom complete data were available. All efforts were made to obtain complete data on each subject regardless of compliance level. Compliance was assessed by pill counting.

The data presented in this thesis 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. That data is presented elsewhere [79]. 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 treatment [21].

Non-parametric tests were used for analyses due to the relatively small sample size and small number of missing data. Despite this, data is given as mean ± standard deviations. Median and interquartile ranges were not used as on further analysis, most data was normally distributed.

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

Carry over effect was assessed by comparing those who had DHEA first to those who had DHEA in the second phase of treatment. Assumptions were made that the two-week washout period was long enough to ensure that there was no such effect.

Potential Problems in Interpretation

It is possible that 12 weeks was not long enough to see these effects, however, previous studies looking at the primary hypothesis of the present study have showed results within 12 weeks.

Missing Data

Data was unable to be collected in some individuals. Of the 33 subjects randomised into the study, one subject withdrew at 10 weeks (on DHEA), one subject withdrew at 11 weeks (on DHEA). 2 subjects withdrew after completing the first half of the study (both on placebo), 1 subject unblinded herself and was excluded from completing the study (on DHEA).

Strength data was not collected in all exercises for all subjects due to a number of reasons. On the occasions where the machines were being serviced (1 occasion at screening for 1 subject), the data for the remaining two visits are analysed in the data. There were subjects who were unable to do specific exercises (1 subject was unable to lie flat for the bicep curls, and another unable to do the chest press due to a rotator cuff injury). These data are excluded from the overall analysis and the presented data is for the remaining subjects.

Results

Of the 33 women who entered the study, complete data was available for 28. Reasons for dropping out are described in the participant's section and shown in Figure 7. Baseline demographic data for the remaining 28 subjects is shown in Table 1.

Baseline Demographics of Study Subjects

Table 1 Overall Baseline Demographic Data

(n = 28)	Mean Baseline Value
Age in years (± SD)	50.25 (15.24)
BMI (Kg/m^2) (± SD)	26.6 (4.37)
Average length of time being hypoadrenal in years (± SD)	12.25 (10.04)
Percentage of postmenopausal women	50 (n = 14)
Percentage of women on oestrogen*	50 (n = 14)
Percentage of subjects with Addison's Disease **	71 (n = 20)
Percentage of subjects with hypothyroidism #	41.9 (n = 13)

* On either oral contraception or oestrogen replacement therapy. ** Other diagnoses include bilateral adrenalectomy due to Cushing's syndrome (n = 6), bilateral benign pheochromocytomas (n = 1) and congenital adrenal hyperplasia (n = 1). Cushing's syndrome cases were due to pituitary adenoma (n = 5), Carney's complex (n = 1). # One 32 year old subject had hyperthyroidism treated by total thyroidectomy aged 16, now on thyroxine replacement therapy.

Hormonal Data

DHEA administration raised levels significantly compared to baseline levels ($p < 0.001$). DHEAS levels were restored to those seen in healthy subjects aged in their late teens – early twenties. Also raised were levels of androstendione and testosterone ($p < 0.001$ for both). These results are shown in Table 2.

Table 2 Hormonal Data

95% Confidence intervals are shown for the differences of the means.

	Mean Levels After 12 weeks on Placebo (± SD)	**Mean Levels After 12 weeks on DHEA (± SD)**	**95% Confidence Intervals**	**p value**
DHEAS (μg/ml)	< 0.3 (0.0)	3.53 (1.30)	2.72, 3.78	< 0.001
Fasting Glucose (mmol/l)	4.83 (0.58)	4.67 (0.54)	-0.42, 0.1	0.054
Fasting Insulin (μU/ml)	8.88 (5.81)	7.07 (4.35)	-3.0, -0.60	0.003
Fasting Glucagon (pmol/l)	56.03 (22.85)	51.06 (17.2)	-9.12, -0.83	0.038
Cortisol (nmol/l)	723 (455)	667 (342)	-180, 68	0.227
Bioavailable Testosterone (%)	10.79 (4.36)	13.32 (5.40)	1.38, 3.69	< 0.001

	Mean Levels After 12 weeks on Placebo (± SD)	**Mean Levels After 12 weeks on DHEA (± SD)**	**95% Confidence Intervals**	**p value**
Androstendione (ng/ml)	0.2 (0.49)	1.69 (1.62)	1.01, 1.97	< 0.001
Sex Hormone Binding Globulin (nmol/l)	60.23 (33.56)	53.66 (34.05)	-12.82, -0.31	0.016
Insulin Like Growth Factor 1 (ng/ml)	245.8 (115.3)	279.2 (101.3)	4.53, 71.75	0.03
Insulin Like Growth Factor 2 (ng/ml)	985.0 (194.8)	938.8 (186.1)	-94.1, 1.5	0.092
Insulin Like Growth Factor Binding Protein 1 (ng/ml)	31.34 (16.80)	28.25 (14.49)	-8.12, 1.92	0.422
Insulin Like Growth Factor Binding Protein 3 (ng/ml)	4779.9 (1316.9)	4841.9 (1473.1)	-477.8, 601.8	0.825

Oestradiol levels in the 5 postmenopausal women not on oestrogen therapy showed a strong trend to being increased on DHEA (20.17 ± 10.1 vs 0.2 ± 0.0 pg/ml, p = 0.063). Levels in premenopausal women were not different (61.5 ± 34.7 vs 66.72 ± 58.21 pg/ml, p = 0.844), but no attempt was made during the study to synchronise with the menstrual cycle.

Insulin Sensitivity - Results

The present study demonstrated significant reductions in fasting plasma insulin and glucagon levels after 12 weeks of DHEA supplementation (7.07 ± 4.35 vs 8.88 ± 5.81 μU/ml, p = 0.003 and 51.06 ± 17.2 vs 56.03 ± 22.85 pmol/l, p = 0.038 respectively). There was a strong trend to lowering fasting glucose (4.67 ± 0.54 vs 4.83 ± 0.58 mmol/l, p = 0.054). This is shown in Figure 14. The M value was higher for DHEA (1.34 ± 0.53 vs 1.22 ± 0.52 mg/min/KgFFM, p = 0.02).

The results of the clamp studies are shown in Figures 12 to 16. Mean insulin levels during the clamps were not significantly different, (45.45 ± 8.01 vs 45.75 ± 10.7 μU/ml, p = 0.824). Mean glucagon levels were significantly lower in the DHEA arm of the study, (38.47 ± 9.14 vs 43.73 ± 11.26 pmol/L, p < 0.001). Glycaemic control was not significantly different between the DHEA and placebo arms (mean glucose 4.88 ± 0.09 vs 4.91 ± 0.12 mmol/l, p = 0.679). However, total volume of 40% dextrose infused was significantly different, (306.0 ± 123.9 vs 274.6 ± 118.1 mls, p = 0.022, and 7.78 ± 3.14 vs 7.06 ± 3.07 mls/KgFFM, p = 0.02) between DHEA and Placebo.

Mean glucose flux was not different between the DHEA and placebo arms (8.00 ± 0.27 vs 7.79 ± 0.26 μmol/KgFFM/min, p = 0.738).

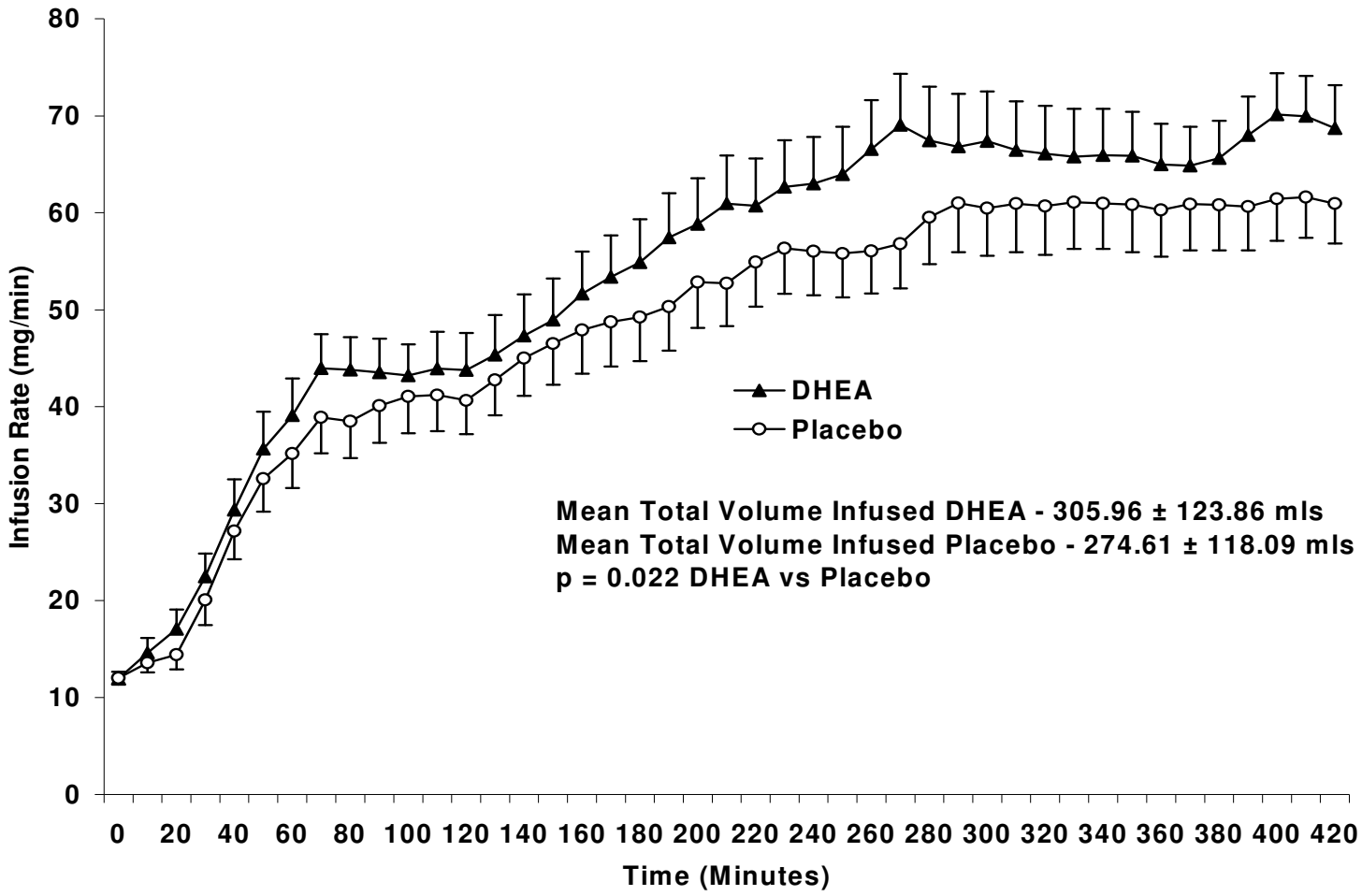


Figure 12 Infusion Rate of 40% Dextrose (mg/min)

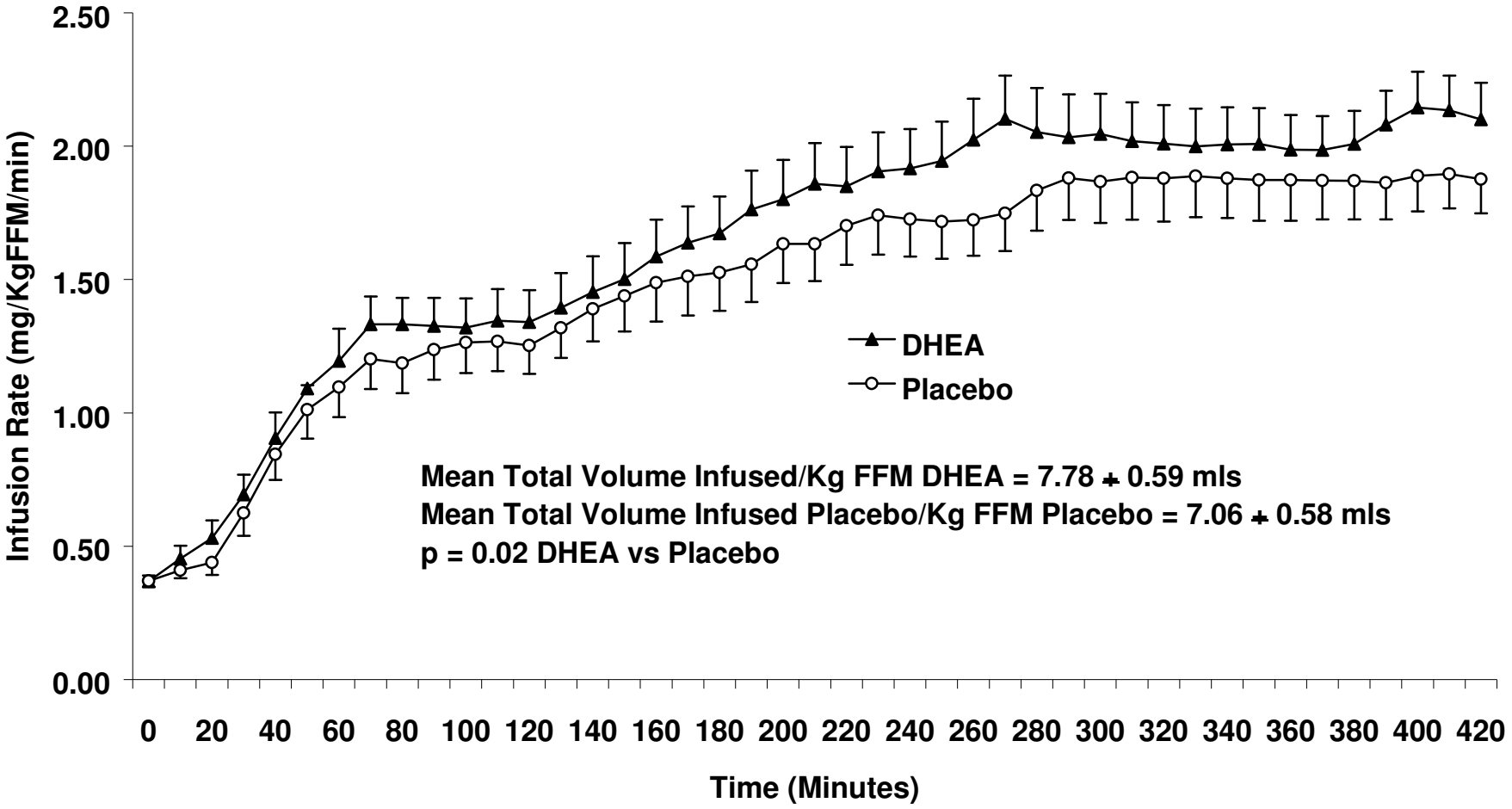


Figure 13 Infusion Rate of 40% Dextrose per Kg FFM

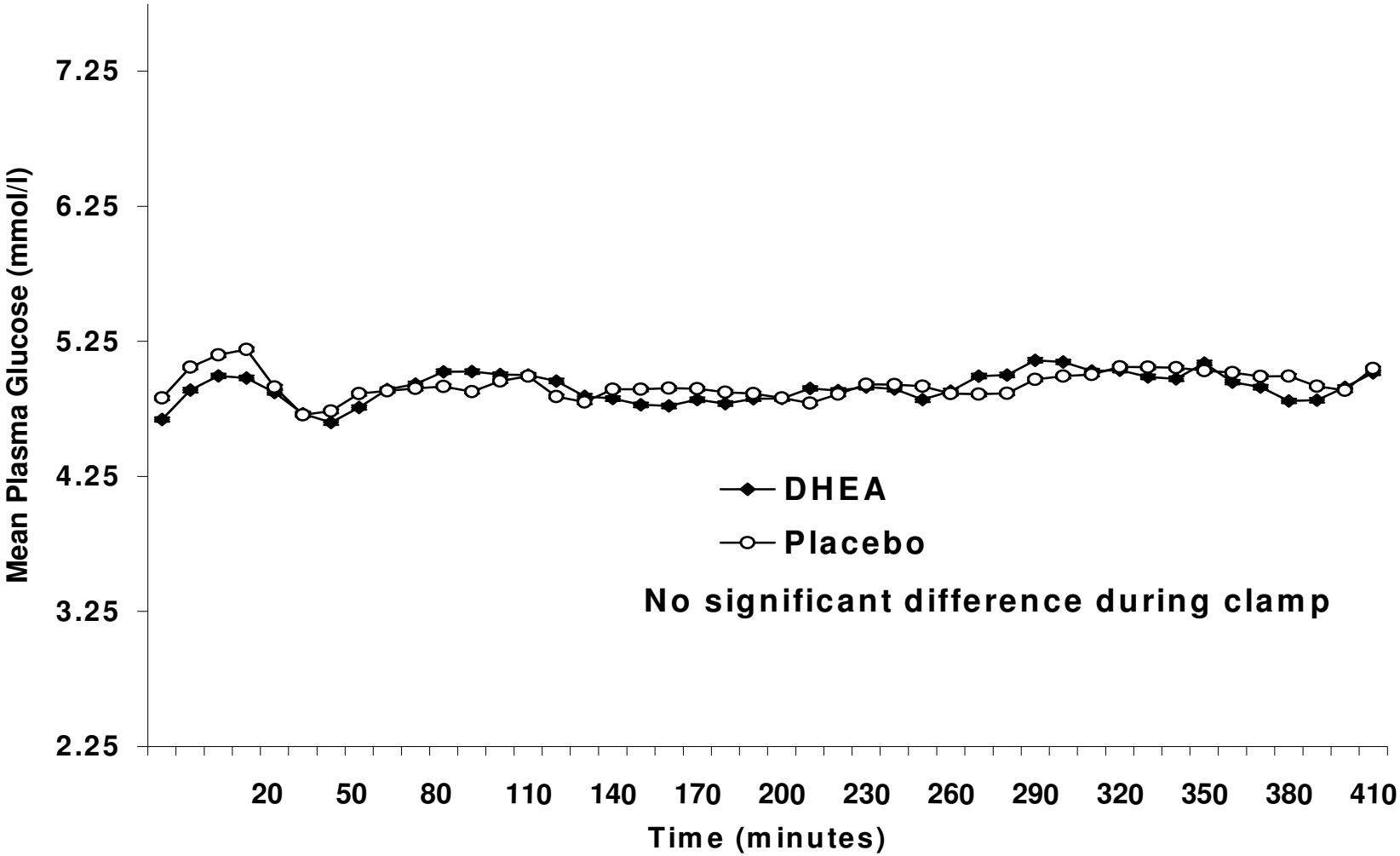


Figure 14 Mean Plasma Glucose During Hyperinsulinaemic Clamp

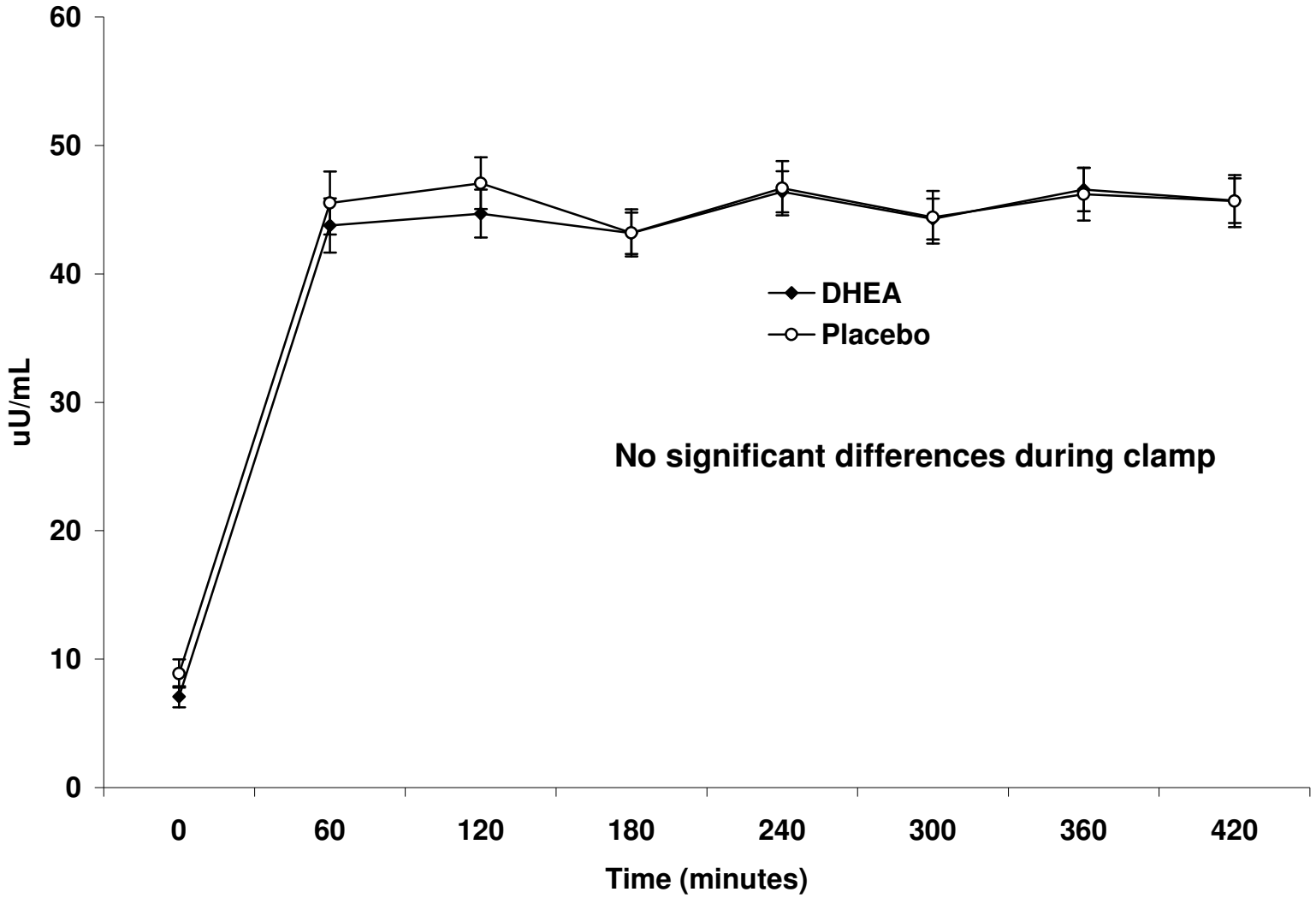


Figure 15 Mean Plasma Insulin Levels During Hyperinsulinaemic Clamp

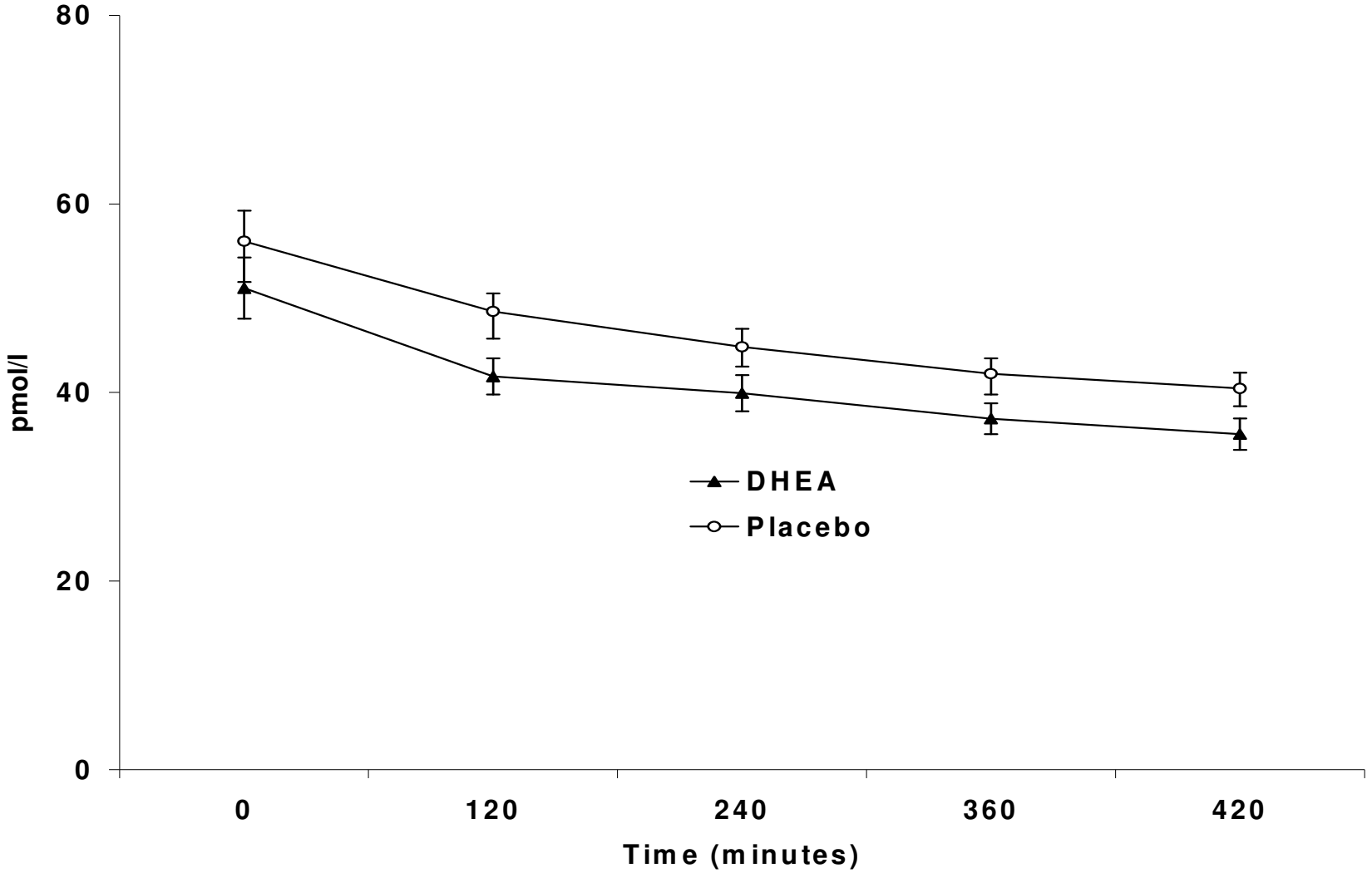


Figure 16 Mean Plasma Glucagon Levels During Hyperinsulinaemic Clamp

Insulin Sensitivity - Discussion

The major new finding of this study is that 50 mg DHEA given once daily to Caucasian hypoadrenal women aged between 20 and 80 years for 12 weeks significantly increased insulin action as measured by a euglycaemic hyperinsulinaemic clamp method.

This is the first study to show an improvement in insulin sensitivity in hypoadrenal subjects given DHEA. Previous studies which have given between 50 mg and 200 mg of DHEA in this group of subjects have not shown significant changes in insulin sensitivity either by measuring fasting insulin levels, doing oral glucose tolerance tests, or euglycaemic clamping [22;74;75;196]. A few studies looking at the effects of DHEA in healthy elderly subjects have shown improvements using indirect measures of insulin sensitivity such as fasting plasma glucose and fasting plasma insulin, or oral glucose tolerance testing [197;198]. However, Diamond et al used a 10% topical formulation of DHEA for 12 months, and while these authors found an 11% reduction in fasting glucose associated with a 17% reduction in fasting insulin, 75g oral glucose tolerance testing results were unchanged between treatment groups.

In a small number of randomised studies with healthy elderly men and postmenopausal women, insulin sensitivity improved significantly [197-199]. Insulin sensitivity was measured indirectly by serum insulin or by euglycaemic clamps. These results are in contrast to studies in women with adrenal insufficiency that have found that either 50 mg or 200 mg per day of DHEA supplementation had no effect on insulin sensitivity [74;196]. Again, the issue arises when comparing the absolute DHEA deficiency in hypoadrenal subjects, and the relative DHEA deficiency in the well elderly. These studies are summarised in Table 3. Insulin sensitivity is currently one of the outcomes

Table 3 Summary of Human Studies Looking at the Effect of DHEA on Insulin Sensitivity.

O = Observational, OL = open label, P = Placebo controlled, R = Randomised trial, C = Cross-over design, N/A = Not applicable, PCOS = Polycystic ovarian syndrome, OGTT = Oral Glucose Tolerance Testing

Type of Subject	Age (years)	Type of Study	Dose (mg/day)	Duration on DHEA	Sex and Number	Summary of Results	Reference
Hyperandro-genic girl	15	OL	300	1 month	F (1)	Marked improvement in insulin sensitivity	Buffington et al [111]
Hyperandro-genic or obese women	18 to 36	OL	1 mg/hr	17 hour infusion	F (5 with PCOS, 5 obese)	No change in insulin sensitivity in either group	Schirock et al [90]
Young Very Overweight Volunteers	Mean age 15.5	P, R	40 sublingual twice daily	8 weeks		No change in insulin sensitivity	Vogiatzi et al [200]
Healthy Overweight Volunteers	21 to 37	P, R	1600	28 days	M (6)	No change in insulin sensitivity	Usiskin et al [201]
Healthy Volunteers	60 to 70	OL	10% cream	12 months	F (15)	Significant improvement in indirect measures of insulin sensitivity but not OGTT	Diamond et al [197]
Healthy Volunteers	64 to 82	OL	50	6 months	M (8) F (10)	Non significant improvement in indirect measures of insulin sensitivity	Villareal et al [162]
Healthy Volunteers	40 to 70	P, R, C	50	3 months	M (13) F (17)	No change in insulin sensitivity	Morales et al [58]

Type of Subject	Age (years)	Type of Study	Dose (mg/day)	Duration on DHEA	Sex and Number	Summary of Results	Reference
Healthy volunteers	40 to 70	P, R, C	50	6 months	M (130) F (17)	No change in insulin sensitivity	Yen et al [60]
Healthy volunteers	22 to 25	P, R	600	28 days	M (10)	No change in insulin sensitivity	Nestler et al [202]
Healthy Volunteers	Mean 56	P, R	25	12 months	F (20)	Significant improvement in insulin sensitivity	Lasco et al [199]
Healthy Volunteers	Mean 57	P, R	1000	30 days	M (22)	Significant improvement in indirect measures of insulin sensitivity	Jakubowicz et al [198]
Hypercholesterolaemic	Mean 54	P, R	25	3 months	M (24)	Significant improvement in insulin sensitivity	Kawano et al [203]
Hypoadrenal	26 to 69	P, R, C	50	3 months	M (15) F (24)	No change in insulin sensitivity	Hunt et al [22]
Hypoadrenal	23 to 59	P, R, C	50	4 months	F (24)	No effect on indirect measures of carbohydrate metabolism	Callies et al [75]
Hypoadrenal	22 to 54	R, P, C	50	9 days	F (10)	No change in insulin sensitivity	Christiansen et al [196]
Hypoadrenal	27 to 51	R	50 or 200	3 months	F (9)	No change in insulin sensitivity	Gebre-Medhin et al [74]

being assessed in long-term DHEA replacement studies of hypoadrenal and well elderly subjects [204;205].

Studies in humans looking at the relationship between insulin sensitivity and DHEA / testosterone ratios have found them to be strongly associated, suggesting that DHEA has an effect on insulin sensitivity [206]. A trial of DHEA infusion in hyperandrogenic women with polycystic ovarian syndrome, however, failed to improve any index of insulin sensitivity – C peptide, basal and post oral glucose tolerance test insulin or any ratio of glucose and insulin. This is in agreement with a more recent study looking at morbidly obese children and young adults given placebo or 40 mg of DHEA sublingually twice daily [200]. These authors also found no association between DHEA administration and changes in any index of insulin sensitivity.

The present study is limited as there is a bias towards obese subjects. The median BMI was 25.9 Kg/m^2, (IQR 23.4, 29.2). Thus this would make the subjects more likely to be insulin resistant. However, as there was still a statistically significant effect on insulin sensitivity, the effect of DHEA replacement on peripheral glucose uptake in hypoadrenal individuals with a normal BMI, without insulin resistance, may be underestimated.

There is some evidence to suggest that DHEA(S) has a role in reducing age-related increases in insulin levels, insulin resistance, and blood glucose [207]. It is difficult to know if this is a cause or effect, but work in humans and in non-human primates has suggested that low serum insulin levels and high DHEA levels are markers of longevity [1]. Taking the alternative view, it has also been demonstrated in observational studies that low DHEAS levels have been associated with hyperglycaemia and insulin resistance [208;209].

Studies looking at the effects of DHEA replacement in healthy humans have been conflicting. Various studies have shown that DHEA improves insulin sensitivity [197-199;203], has no effect [58;60;165;200;202], or worsens it [210]. Jakubowicz et al studied 22 healthy men with a mean age of 57 years, using 100 mg DHEA per day for 30 days. In this group, serum insulin decreased from 35.3 to 25.8 mU/ml, while serum glucose declined from 5.15 to 4.90 mmol/l [198]. Serum insulin and glucose did not change significantly in the placebo group. However, no formal tests of insulin sensitivity were done. Kawano et al reported an improvement in insulin sensitivity measured by a fasting glucose measurement in 24 hypercholesterolaemic but otherwise healthy men aged 54 ± 1 year after 12 weeks of 25 mg of oral DHEA [203]. This is illustrated in Figure 17. There was no change in fasting insulin levels compared with placebo.

The results of the present study, therefore, may be important in other groups of relatively hypoadrenal subjects, such as those in intensive care, because it is in this group that adrenal insufficiency is common [211], and in whom insulin therapy has been shown to improve outcomes [212].

The lack of difference in fasting glucose flux between DHEA and placebo is not surprising since fasting glucose concentrations were normal.

The results of the present study suggest that these subjects were insulin resistant. Their M values were lower than those found previously in healthy volunteers [213;214]. This may suggest that, whilst these subjects were biochemically normal, it may have been that the dose of corticosteroid replacement may have been too high, leading to steroid induced insulin resistance. This issue remains to be explored.

It is interesting to speculate about the possible mechanisms for the improved insulin sensitivity seen with DHEA replacement in hypoadrenal women. As there were no increases in mitochondrial enzyme activity it is unlikely that there was an increase in mitochondrial ATP production with possible uncoupling of the oxidative phosphorylation pathway. However, this remains to be confirmed. In addition, there were no changes seen in body composition, in particular in lean body mass, which may account for an increase in insulin sensitivity. However, intramyocyte triglyceride levels were not measured. This has been previously been shown to be a cause of increased insulin resistance [215].

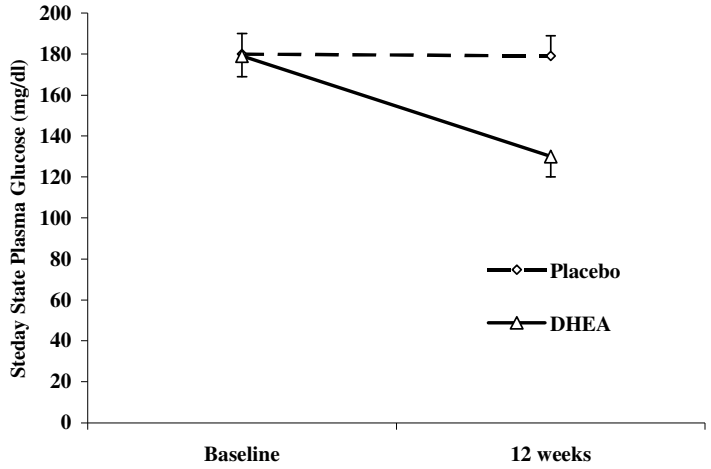


Figure 17 Change in Insulin Sensitivity with DHEA vs Placebo

In 24 healthy hypercholesterolaemic men ($p < 0.01$). From reference 203

One possible explanation is that there was an increase in glucose uptake transporter expression within skeletal muscle. Studies have shown that the abundance of transcripts encoding GLUT 1 increased when fibroblasts were cultured with insulin and DHEA [216]. The in vitro results looking at GLUT expression have not been reproduced in vivo [83;217]. A study by Nestler et al showed that hyperinsulinaemia reduced serum DHEA(S) levels [218], but no potential mechanisms for this phenomenon were discussed.

Recent evidence has provided clues as to the possible mechanisms for this protection. DHEA has been shown to increase phosphatidylinositol 3-kinase, thus increasing muscle insulin signalling [83], and protein kinase C activity, thus increasing insulin mediated glucose uptake [219], or DHEA may mimic atypical protein kinase C activity, thus acting in such a way to prevent or reduce the effect of steroid induced insulin resistance [220].

Recent work has looked at the effects of supraphysiological doses of DHEA on glucose uptake in human fibroblast cell lines. This has shown that glucose uptake is significantly increased [216].

Furthermore, another study showed that the addition of supraphysiological doses of insulin increased the sensitivity of the glucose uptake to DHEA [216]. This may be related to the increase in IGF 1 levels, as previous work has shown low IGF 1 levels in elderly subjects to be associated with decreased insulin sensitivity when measured by the euglycaemic clamp [221].

Aoki et al have followed up the work of Coleman et al [61] looking at the effects of DHEA on the hepatic gluconeogenic enzymes such as glucose - 6 - phosphatasase [222]. Theses authors have shown that DHEA works in a similar manner as the thiazolidinediones, and reduces the levels of these enzymes in insulin resistant mice. This lead to an increase in insulin sensitivity when assessed by euglycaemic hyperinsulinaemic clamping. As in the present study, these authors also found that DHEA administration was found to lower fasting insulin levels.

In summary, this is the first study to show that 12 weeks of supplemental DHEA is associated with an increase in insulin mediated glucose uptake in hypoadrenal women.

There is some justification for the continued availability of this hormone, but that further work is needed before the use of DHEA can be routinely recommended in hypoadrenal subjects.

Insulin Like Growth Factors - Discussion

As shown in Table 2, IGF 1 levels rose significantly whilst on DHEA ($p = 0.03$).

The present study confirms that of others showing that DHEA replacement increases IGF 1 levels in hypoadrenal individuals [21;223]. However, this effect has not been seen in all studies [80;224]. Whether the decline in IGF 1 levels seen with ageing is due to a decline in DHEA levels, is not known. It is difficult to know if DHEA exhibits a 'permissive' effect on IGF 1, i.e. if by having high DHEA levels, IGF 1 levels are also maintained. Studies in ageing subjects have also had conflicting results. Some observational work has shown no link between DHEA levels and IGF 1 levels [225;226], with other authors stating there is an association [227;228]. However, interventional studies in older subjects have shown increases in IGF 1 levels [58;162;165;229].

The increase in IGF 1 levels has been put forward as one of the reasons for the improvement in well being with DHEA(S) replacement [58;60;164]. In one study this increase in IGF 1 was restricted to those women with primary adrenal insufficiency [21], suggesting that the effect of DHEA(S) on IGF 1 production is growth hormone dependent.

IGF BP1

In elderly subjects the levels of IGF BP1 are not significantly related to the development of coronary artery disease [230], however levels decreased significantly with DHEA(S) replacement therapy [58;60] resulting in a raised IGF 1 / IGF BP1 ratio suggesting increased bioavailability of IGF 1 to target tissues. As mentioned in the section in IGF 1, this may have an effect on cardiovascular outcomes, but this work has yet to be done.

Lipids – Results

The present study demonstrated significant reductions in total cholesterol, (5.23 ± 0.83 vs 4.62 ± 0.9 mmol/l, p = 0.007), triglycerides, (1.52 ± 0.7 vs 1.70 ± 0.80 mmol/l, p = 0.016), and HDL cholesterol, (0.97 ± 0.32 vs 1.09 ± 0.33 mmol/l, p = 0.003) on DHEA vs placebo. The Total Cholesterol / HDL cholesterol ratios were not different between the two arms of the study (4.76 ± 1.7 vs 4.62 ± 1.8, p = 0.293 DHEA vs placebo)

These findings confirm those of other authors, and are discussed within this context below.

Lipids – Discussion and the Cardiovascular Effects of DHEA

Animal studies have shown that administration of DHEA reduces the build up of atherosclerotic plaque in animals fed a high fat diet [67;68]. In addition, DHEA has been shown to reduce platelet adhesion in vivo [69]. Thus, an increase in plaque formation may be an explanation for the increase in cardiovascular events in those with low DHEA levels [63;231]. In addition, Nestler et al showed that hyperinsulinaemia reduces serum DHEA levels [232]. As insulin is thought to be proatherogenic, a reduction in circulating insulin levels is a possible explanation for the antiatherogenic actions of DHEA [63;67-69;202;231]. This is despite the reduction in total cholesterol seen in the present study and by several other authors [21;199;202]. The associated reduction in HDL seen in the present study has also been shown by other authors [233], but not all [165;199]. Although the hypothesis that DHEA levels are inversely correlated with cardiovascular disease rates remains to be formally tested in human studies, there are some animal data and human epidemiological

data that suggest that this is the case [63;67-69;202]. Table 4 summarises much of the current data.

The epidemiological evidence in humans is conflicting, as some studies demonstrate an inverse relationship between DHEA levels and increased cardiovascular risk in men, but not in women [63;64;234-236]. In a large ongoing observational study looking at cardiovascular risk factors, diabetes in men over 50 was associated with a low DHEA [208]. However, this relationship is not seen in all large epidemiological studies [70].

One study looking at cardiovascular mortality in ageing men followed for 12 years after a single DHEA(S) measurement showed that those subjects with a history of cardiovascular disease at enrollment had a significantly lower DHEA(S) level than in those without such a history [63]. Whilst their conclusions were limited to a single determination of DHEA(S) levels, the data suggested that the DHEA(S) concentration is independently and inversely related to death from any cause and death from cardiovascular disease in men over age 50. The same authors conducted a similar study in women and failed to find any significant relationship between DHEA(S) levels and either cardiovascular or overall mortality [235].

DHEA may affect various other risk factors. In women there was also a positive relationship between DHEA levels and the development of glucose intolerance [237;238]. This conflicts with the data from the current study, but these data come from observational long term longitudinal and observational studies, and thus from a population different from that used in the present study.

Further difficulties in interpreting this data come from evidence that shows that DHEA administration lowers HDL levels [164].

There is some evidence to suggest a mechanism for the observed reduction in triglycerides. The antiobesity effect of DHEA in rats may be due to a combination of elevated mitochondrial respiration [131;140], and also lowered insulin levels. More recent evidence has emerged to suggest that DHEA may have a direct effect on adipocytes, by down regulating the expression of peroxisome proliferator - activated receptor γ receptors [94]. This resulted in lower triglyceride levels. This is thought to be through enhancement of beta-oxidation rates, which have been inferred by the increase in acyl-CoA-oxidase activity and of cytochrome P450 4A content [141]. This reduction in expression may be significant, as similar changes in differential expression of peroxisome proliferator - activated receptor γ isoforms have resulted in reductions in obesity and insulin resistance in a human population [94;239].

A recent randomised double blinded placebo controlled study was done looking at the effects of 25 mg of DHEA given for 12 weeks in 24 hypercholesterolaemic men [203]. This looked at vascular endothelial vasodilatation in the brachial artery after transient occlusion. These authors showed a statistically significant increase in nitric oxide dependent vasodilatation following DHEA administration. They suggest that an increase in cardiovascular disease seen with ageing is due to the loss of protection afforded by high circulating DHEA(S) levels. These changes are shown in Figure 18. This finding is consistent with animal work looking at DHEA preventing pulmonary vasoconstriction in chronically hypoxic rats [240]. This work suggested a further mechanism for the beneficial effects of DHEA on vascular smooth muscle cells [240]. DHEA administration seems to block the activity of potassium dependent calcium entry into vascular smooth muscle, preventing contraction, thus preventing vasoconstriction.

Free and total IGF 1 levels are lower in individuals with coronary artery disease [230;241] and total IGF 1 are lower in subjects with an atherogenic lipid profile [242]. In addition, low IGF 1 levels in the elderly are associated with lower insulin mediated glucose uptake [221]. This effect may lead to an increased predisposition for the development of diabetes mellitus. Studies assessing IGF 1 treatment have shown a reduction in total cholesterol, low-density lipoprotein and very low-density lipoprotein [243]. In addition, IGF 1 may have an important regulatory function in nitric oxide dependent vascular endothelial relaxation [244]. This may be either via a cell membrane bound receptor, which activates tyrosine kinase which in turn initiates a series of intracellular processes via a signal transduction pathway [245], or via the recently described G protein coupled receptor for DHEA on vascular endothelial cells [36]. All of these factors are known to be important in the development of cardiovascular disease suggesting that a rise in IGF 1 levels may be of benefit. Many of these findings, such as the beneficial changes in lipid profile, nitric oxide dependent endothelial relaxation, mirror those finding seen in some animal and human studies after increasing circulating levels DHEA [199;202;203].

Decreased IGF 1 levels seen in some individuals may lead to a reduction in the promotion of vascular nitric oxide production, which may be a mechanism by which cardiovascular symptoms may become apparent [246]. It is difficult to know if this is a cause of the increased cardiovascular disease or occurs as a result of it, but as low IGF 1 levels, as well as lowered DHEA(S) levels, are seen in sub clinical disease it remains a possibility that the proatherogenic milieu is promoted by the reduction in the protective

actions of both DHEA(S) and IGF 1 [247]. Overall, the role of IGF 1 and DHEA(S) in the prevention of the development of cardiovascular disease remains to be fully elucidated.

The present study showed that whilst there was a reduction in total cholesterol HDL, and triglycerides, because the total chosterol/HDL cholesterol ratio was unchanged – and thus the effect on cardiovascular risk neutral, DHEA replacement may have an overall beneficial effect on cardiovascular risk because of the reduction in triglycerides.

Currently there are no data from long term interventional trials looking at the effects of DHEA supplementation on cardiovascular outcomes. As the numbers required for such a study are probably very large, it is unlikely that this will ever be conducted.

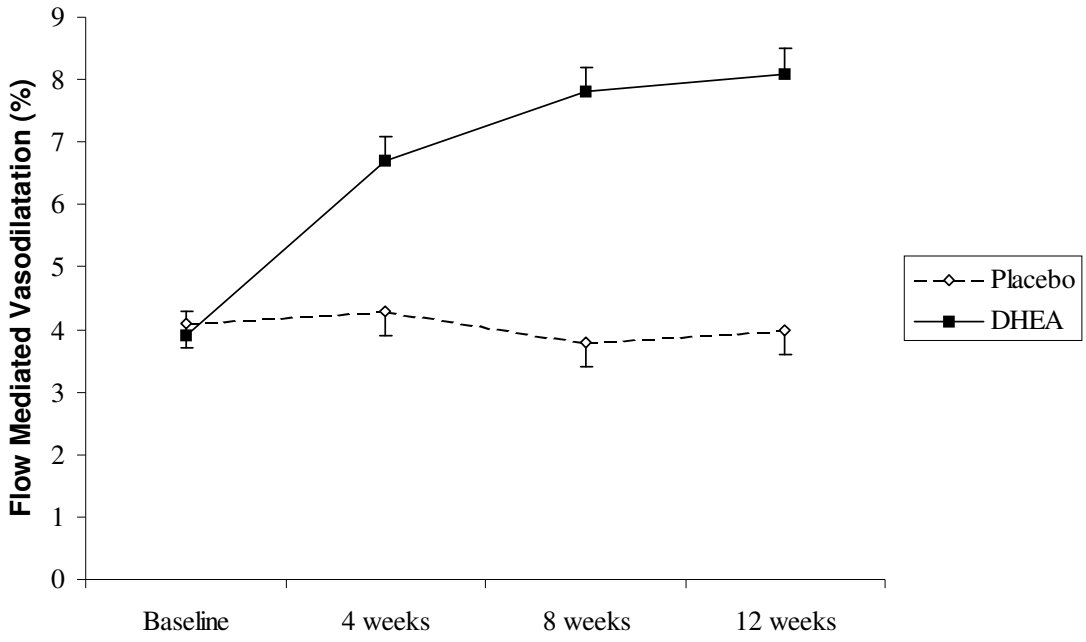


Figure 18 Effects of 12 Weeks of 25 Mg DHEA Supplementation on Flow Mediated Endothelial-Dependent Dilatation of the Brachial Artery

$P < 0.01$ at all points except baseline. From reference 195

Table 4 Summary of Human Studies Looking at the Effect of DHEA on Cardiovascular Outcomes.

O = Observational, N/A = Not Applicable, MI = Myocardial Infarction

Type of Subject	Age (years)	Type of Study	Dose (mg/day)	Duration on DHEA	Sex and Number	Summary of Results	Reference
Healthy volunteers	50 to 79	O	N/A	N/A	M (242)	Low DHEA levels associated with higher death rates	Barrett-Connor et al [63]
Healthy volunteers	40 to 70	O	N/A	N/A	M (1709)	Low DHEA levels associated with higher rates of cardiovascular disease	Feldman et al [236]
Healthy volunteers	65 to 76	O	N/A	N/A	M (963) F (1171)	In men, highest mortality in the lowest DHEA quartile. In women highest mortality in the highest DHEA quartile.	Trivedi et al [248]
Healthy volunteers	60 to 79	O	N/A	N/A	F (289)	High DHEA levels associated with higher death rates	Barrett-Connor et al [64]
Healthy volunteers	Mean 65	O	N/A	N/A	F (942)	DHEA levels not associated with fatal cardiovascular outcomes	Barrett-Connor et al [235]
Post MI	26 to 40	O	N/A	N/A	M (32 cases, 76 controls)	Significantly lower DHEA levels found in post MI subjects	Slowinska-Srzednicka et al [231]

Body Composition and Strength Data - Results

There were no changes in any measured parameter of body composition or strength. These results are shown in Table 5 and Table 6. After initial analysis of DEXA results, and the lack of any positive findings, a decision was made not to analyse the deuterated water samples.

Table 5 Body Composition Data

95% Confidence intervals are shown for the differences of the means. n = 28

	Mean Levels After 12 weeks on Placebo (± SD)	**Mean Levels After 12 weeks on DHEA (± SD)**	**95% Confidence Intervals**	**p value**
Fat Free Mass (Kg)	39.67 (4.69)	39.81 (4.56)	-0.93, 1.84	0.756
Fat Mass (Kg)	29.36 (8.99)	29.13 (9.64)	-3.81, 0.70	0.262
Bone Density (g/cm^2)	1.18 (0.10)	1.17 (0.10)	-0.01, 0.01	0.748

Table 6 Strength Test Results

95% Confidence intervals are shown for the differences of the means. n = 28 unless otherwise stated.

	Mean Levels After 12 weeks on Placebo (± SD)	**Mean Levels After 12 weeks on DHEA (± SD)**	**95% Confidence Intervals**	**p value**
Grip Strength (lbs)	64.51 (12.87)	63.92 (13.01)	-2.97, 2.15	0.7
Biceps Curl (lbs) n = 27	82.41 (15.15)	82.96 (16.94)	-2.31, 3.42	0.72
Leg Press (lbs)	127.07 (34.86)	126.16 (32.56)	-4.96, 5.14	0.72
Chest Press (lbs)	87.96 (18.89)	89.07 (20.1)	-1.98, 4.21	0.45
Leg Curl (lbs)	87.04 (24.78)	87.59 (24.82)	-2.42, 3.53	0.71

Body Composition and Strength Data - Discussion

The present study failed to find any differences in strength or body composition during the DHEA phase of the study. This is in agreement with several other interventional studies in healthy subjects and hypoadrenal women which have also failed to show any changes in body composition with DHEA administration [58;74;75;200;201;210;249;250]. This is in conflict with other work showing that DHEA does alter body composition [162;165;197;202].

Animal studies have shown that DHEA in the diet can either prevent weight gain, or even cause weight loss [82;87;119;251]. The proposed mechanisms for this have been discussed in the section looking at the effects of DHEA on mitochondria.

Some observational studies in healthy volunteers trying to correlate DHEA levels to muscle strength and body composition, have failed to find any association [226], while other data in obese individuals of varying ages have found a positive relationship [209;252]. However, in both of these latter studies body composition was estimated by anthropomorphic measures only, while the studies that showed no association used Dual X-Ray Absorptiometry (DEXA).

Studies have also been done in healthy young individuals as well as the healthy elderly. Oral administration of DHEA (1600 mg/d) for four weeks was reported to decrease fat mass by an average of 3.8 kg and increase fat-free body mass by an average of 4.5 kg [202]. However, these results differ from those of Welle et al, who failed to demonstrate a similar effect on administering 1600 mg/d of DHEA in 8 men aged 23 to 43 years old [253]. In another study, DHEA administration (50 mg/d) to elderly men and women increased the bioavailability of IGF 1 by 50%. This was achieved by a 10%

increase in total IGF 1 with a 19% decrease in IGF BP1 levels [58]. An increase in IGF 1 level and bioavailability has been reported with 100 mg/d of DHEA in both men and women [60]. In their study of elderly men and women given DHEA for several weeks, Yen et al also demonstrated a significant increase in lean body mass measured by DEXA scan. Muscle strength showed an improvement only in men, because women showed a strong response to placebo. Fat mass decreased only in men. This study involved only 8 men and 8 women with 6 months of DHEA administration. Compliance with DHEA intake was not monitored by regular DHEA(S) measurements, but both older men and women reached DHEA(S) levels comparable to those of young men. At these relatively high levels for women, it was reported that at least some of the women tended to develop increased facial hair, whereas men had no side effects.

A more recent randomised double blind placebo controlled study looked at a 50 mg replacement dose of DHEA for 1 year in 280 men and women between the ages of 60 and 80 [254]. This study failed to show any improvement in handgrip strength, knee muscle strength, or thigh cross sectional area despite restoration of DHEA(S) levels to that seen in younger individuals.

Most of the studies to measure body composition were performed after a short period (4 to 6 weeks) of administration of DHEA(S). It is unlikely that any of the techniques used to assess body composition, such as DEXA or total body water, are sufficiently sensitive to detect the small changes in lean body mass likely to occur over such a short period of time. As described above, the study by Yen et al, demonstrated a significant increase in fat free mass when DHEA was administered for six months in 8 men and 8 women [60]. This increase appears to be approximately 1.5 kg in lean mass in a

75 Kg person. These variables are not well described in the published report, and other sensitive and specific parameters of muscle mass (e.g. CT scan) were not applied to confirm that muscle mass increases on DHEA(S) administration. The current literature is summarised in Table 7.

Morales et al showed that a 100 mg dose of DHEA for 6 months was associated with a reduction in fat mass in well elderly men, but not in women [165]. Additionally, the study by Diamond et al showed that topical application of a 10% cream of DHEA for 1 year was associated with a statistically significant reduction in femoral fat, an increase in femoral muscular area, and decreased skinfold thickness [197]. These findings are consistent with a smaller study by Nestler et al, which found a change in body composition in a small number of healthy young men given a highly suprapharmacological dose of 1600 mg of DHEA for 28 days [202]. This study showed that there was a decrease in body fat, with an overall increase in skeletal muscle tissue. Villareal et al showed that a 50 mg dose of DHEA for 6 months was associated with a statistically significant reduction in body fat and increase in lean mass in 18 healthy elderly men and women [162].

Muscle strength in healthy elderly volunteers has been assessed. In observational studies, quadriceps strength has been positively correlated to circulating DHEA levels in men [72;250], but not women [250]. This correlated with the findings of the interventional study by Morales et al, where there was an increase in quadriceps and lumbar strength in men but not in women [165]. These studies are summarised in Table 7.

The study by Morales et al showed a statistically significant rise in IGF 1 levels [165]. Epidemiological work has shown that high IGF 1 levels have been correlated with maintenance of fat free mass (or, a reduction in the rate of loss of lean mass), as well as

reduction in the rate of development of sarcopaenia [255]. In the present study, 50 mg/d of DHEA was also associated with a statistically significant 12.2% increase in IGF 1, and an 11% decline in IGF BP1 levels. Despite these changes being similar to the 10% increase in total IGF 1 with a 19% decrease in IGF BP1 levels seen by Morales et al [58], no change in body composition was seen. The study did show that in men, but not in women, fat body mass decreased significantly, ($p = 0.02$) and knee muscle strength also significantly increased ($p = 0.02$), as well as an increase in lumbar back strength ($p = 0.01$). The present study is in agreement with several others which have shown that levels of IGF BP3 remain unchanged with DHEA administration [58;60;162].

In general it appears that long-term studies (i.e. those lasting greater than 4 weeks) tend to show changes in body composition and muscle strength. Long-term placebo controlled double blind studies are needed to determine the effects of DHEA on body composition and muscle.

Table 7 Summary of Human Studies Looking at the Effect of DHEA Replacement on Muscle Strength and Body Composition.

O = Observational, OL = open label, P = Placebo controlled, R = Randomised trial, C = Cross-over design, N/A = Not applicable

Type of Subject	Age (years)	Type of Study	Dose (mg/day)	Duration on DHEA	Sex and Number	Summary of Results	Reference
Healthy Volunteers	21 to 96	O	N/A	N/A	M (578)	Positive association between DHEAS levels and muscle strength in men above 56 years old	Valenti et al [72]
Healthy Volunteers	60 to 70	OL	10% cream	12 months	F (15)	Decrease in femoral fat, increased femoral muscle. Decrease in skinfold thickness	Diamond et al [197]
Healthy Volunteers	64 to 82	OL	50	6 months	M (8) F (10)	Significant decrease in fat mass with significant increase in fat free mass	Villareal et al [162]
Healthy, Young, Very Overweight Volunteers	Mean age 15.5	P, R	80 (40 sublingual twice daily)	8 weeks		No change in body weight, body composition, or serum lipids	Vogiatzi et al [200]
Healthy Overweight Volunteers	21 to 37	P, R	1600	28 days	M (6)	No change in anthropological measurements of body composition	Usiskin et al [201]
Healthy Volunteers	50 to 65	P, R, C	100	6 months	M (9) F (10)	Decrease in fat mass in men only. Increase in knee and lumbar strength in men only	Morales et al [165]

Type of Subject	Age (years)	Type of Study	Dose (mg/day)	Duration on DHEA	Sex and Number	Summary of Results	Reference
Healthy Volunteers	40 to 70	P, R, C	50	3 months	M (13) F (17)	No change in body composition	Morales et al [58]
Healthy Volunteers	46 to 61	P, R, C	1600	28 days	F (60)	No change in body composition	Mortola et al [210]
Healthy Volunteers	60 to 84	P, R, C	100	3 months	M (39)	No change in body composition	Flynn et al [249]
Healthy volunteers	22 to 25	P, R	600	28 days	M (10)	31% decrease in body fat in 4 out of 5 subjects (p value not given)	Nestler et al [202]
Anorexic	14 to 28	R	50	12 months	F (61)	Significant weight gain	Gordon et al [256]
Hypoadrenal	22 to 54	R, P, C	50	9 days	F (10)	No change in body composition	Christiansen et al [196]
Hypoadrenal	26 to 69	P, R, C	50	3 months	M (15) F (24)	No change in body composition	Hunt et al [22]
Hypoadrenal	27 to 51	R	50 or 200	3 months	F (9)	No change in body composition	Gebre-Medhin et al [74]

The Effects of DHEA on Bone

Rat studies have shown that DHEA reduces the rate of bone loss usually seen after oophorectomy [257]. There is conflicting evidence concerning the influence of DHEA on bone turnover in humans. It has been demonstrated that gonadal androgen deficiency is associated with osteoporosis in men [258]. This is despite normal adrenal function, thus it can be inferred that in men, androgens derived from adrenal precursors are insufficient to maintain normal bone architecture. It is also clear from individuals with receptor defects or enzyme deficiencies that in men, oestrogens are also necessary for normal bone maturation and formation [259;260;260;261].

In women, ageing is associated with a decline in oestrogens and adrenally derived androgens. This decline may be causally associated with the development of osteoporosis, as DHEA is converted to oestrone within osteoblast - like cells by aromatase cytochrome P450 in culture [262;263]. Thus, DHEA may contribute to the maintenance of BMD in postmenopausal women. In addition, there is cross sectional and longitudinal data to show that there is a correlation between DHEA levels and lumbar BMD [264;265]. However, these findings are in disagreement with long-term work that has shown no such relationship in either men or women [266]. This negative finding mirrored those of interventional studies of healthy subjects that showed DHEA supplementation having no effect on markers of bone turnover or on BMD measured by DEXA [80;164;267;268]. However, open label studies have shown that DHEA made a significant difference in BMD in healthy elderly subjects [162;269].

In untreated hypoadrenal subjects there is evidence that bone loss develops. This is perhaps because of the permissive effects of cortisol on osteoblast and osteoclast

recruitment and activation, and perhaps due to the permissive effects of cortisol on skeletal growth and maturation during earlier years in life. It is suspected that unrecognised or untreated significant hypoadrenalism, especially during critical times of skeletal development, could have an impact on the adult bone density. Hypocortisolaemia might effect gonadal steroid secretion centrally or peripherally, so it is difficult to know which is the true proximal cause of low bone density in these patients. It is likely, however, that most cases of osteoporosis seen in treated subjects are due to over replacement with glucocorticoids.

There is some evidence of uncertain significance from one study showing that DHEA replacement in hypoadrenal subjects is useful [75]. In this study serum osteocalcin was significantly raised, but there was no corresponding increase in the excretion of urinary cross-links. This finding has not been seen in other interventional studies of hypoadrenal subjects [22;224].

The present study did not show and significant changes in bone density. This is not surprising as the study was not powered to show any significant effect of DHEA replacement on bone density in hypoadrenal women with only 3 months of DHEA replacement. However, bone turnover markers were not measured and may have been altered. As bone turnover is slow, a large study currently underway at Mayo Clinic, Rochester, MN is assessing the effects of 50 mg of DHEA replacement daily for 2 years on bone density and bone turnover.

Thus the issue of DHEA in the prevention of osteoporosis in elderly or hypoadrenal subjects remains an area of uncertainty. The current literature regarding the relationship between DHEA and bone is summarised in Table 8.

Table 8 Summary of Human Studies Looking at the Effects of DHEA Replacement on Bone.

O = Observational, OL = open label, P = Placebo controlled, R = Randomised trial, C = Cross-over design, N/A = Not applicable,

BMD = Bone Mineral Density

Type of Subject	Age (years)	Type of Study	Dose (mg/day)	Duration on DHEA	Sex and Number	Summary of Results	Reference
Healthy Volunteer	50 to 74	O	N/A	N/A	M (260) F (162)	No correlation between DHEA levels and BMD at any site	Barrett-Connor et al [266]
Healthy Volunteer	(Not given) "Postmeno pausal"	P, R	25	6 months	F (13)	No effects on bone turnover markers or BMD	Casson et al [164]
Healthy Volunteer	60 to 79	P, R, C	50	12 months	M (140) F (140)	In men no effects on bone turnover markers or BMD In women positive effect in BMD at several sites	Baulieu et al [270]
Healthy Volunteer	50 to 69	P, R, C	50	4 months	M (22)	No effects on bone turnover markers	Arlt et al [267]
Healthy Volunteer	56 to 80	P, R	90	6 months	M (43)	No effects on bone turnover markers	Kahn et al [268]
Healthy Volunteer	60 to 70	OL	10% cream	12 months	F (14)	Significant increase in BMD and significant decrease in bone turnover markers	Labrie et al [269]

Type of Subject	Age (years)	Type of Study	Dose (mg/day)	Duration on DHEA	Sex and Number	Summary of Results	Reference
Healthy Volunteers	64 to 82	OL	50	6 months	M (8) F (10)	Significant increases in total body and spinal BMD	Villareal et al [162]
Anorexic	14 to 28	R	50	12 months	F (61)	Significantly reduced levels of the bone resorption markers. Non significant maintenance of hip and spinal BMD	Gordon et al [256]
Hypoadrenal	23 to 59	P, R, C	50	4 months	F (24)	Increase in serum osteocalcin with no change in urinary cross-link excretion	Callies et al [75]
Hypoadrenal	26 to 69	P, R, C	50	3 months	M (15) F (24)	No effects on bone turnover markers or BMD	Hunt et al [22]
Hypoadrenal	24 to 70	P, R	25	9 months	F (39)	No effects on bone turnover markers	Løvås et al [80]
Hypoadrenal	25 to 65	R (OL)	30 (<45 years old), 20 (≥ 45 years old)	6 months	F (38)	No effects on bone turnover markers or BMD	Johannsson et al [224]

Exercise Capacity and Physical Function – Results

The current study showed that 12 weeks of DHEA replacement resulted in no changes in any measurement of physical function. These results are shown in Table 9.

Table 9 Exercise Capacity and Physical Function Tests Results

95% Confidence intervals are shown for the differences of the means.

	Mean Levels After 12 weeks on Placebo (± SD)	**Mean Levels After 12 weeks on DHEA (± SD)**	**95% Confidence Intervals**	**p value**
Indirect Calorimetry VCO_2 (mls/min)	170.18 (20.2)	170.5 (20.45)	-8.0, 8.64	0.85
Indirect Calorimetry VO_2 (mls/min)	205.07 (27.04)	204.96 (24.92)	-7.33, 7.12	0.93
Respiratory Quotient	0.833 (0.064)	0.834 (0.056)	-0.031, 0.033	0.67
Resting Energy Expenditure per Kg FFM (kcal/day/KgFFM)	36.06 (4.4)	35.75 (3.34)	-1.5, 0.89	0.625

	Mean Levels After 12 weeks on Placebo (± SD)	**Mean Levels After 12 weeks on DHEA (± SD)**	**95% Confidence Intervals**	**p value**
Peak Bike Power (Watts)	119.81 (35.67)	115.89 (34.37)	-8.11, 1.45	0.2
Peak VO_2 (mls/min)	1450 (338)	1445 (321)	-59, 51	0.92
Peak VO_2 per Kg Body Weight (mls/min/Kg Body Weight)	20.36 (5.32)	20.37 (5.02)	-0.64, 0.67	0.97
Peak VO_2 per Kg FFM (mls/min/Kg FFM)	36.7 (8.44)	36.34 (7.42)	-1.84, 1.12	0.79
Peak VCO_2 (mls/min)	1707 (417)	1718 (406)	-57, 76	0.79
Peak Heart Rate (beats per minute)	160 (21)	161 (22)	-1, 9	0.21

	Mean Levels After 12 weeks on Placebo (± SD)	**Mean Levels After 12 weeks on DHEA (± SD)**	**95% Confidence Intervals**	**p value**
Peak VE (l/min)	63.89 (16.02)	62.38 (15.66)	-4.14, 1.11	0.36
Peak RER	1.178 (0.091)	1.187 (0.079)	-0.012, 0.031	0.36

Exercise Capacity and Physical Function - Discussion

The current study has shown that 12 weeks of DHEA given to hypoadrenal women has no effect on exercise capacity or physical function. This is in agreement with previous studies done in elderly subjects given DHEA [267;271]. Very little work has been done looking at this aspect of physical capacity in hypoadrenal women. With the inconsistent reports on changes in body composition, it is difficult to know if any changes in lean body mass are matched to changes in physical (aerobic) capacity. This is discussed a little more in the section on skeletal muscle protein synthesis rates and enzyme activity.

Skeletal Muscle Protein Synthesis Rates – Results

In the 7 subjects in whom synthesis rates were measured, there were no changes in the fractional synthesis rates of mitochondrial or sarcoplasmic proteins during the DHEA arm of the study, when compared to placebo. These results were confirmed by the use of 3 different substrates – ^{15}N Phenylalanine, ^{15}N Tyrosine, or D_4 Tyrosine. There were no differences in the calculated results using any of these tracers (data not shown). The results using ^{15}N Phenylalanine as the precursor are shown in Table 10.

Table 10 Mitochondrial and Sarcoplasmic Protein Fractional Synthesis Rates

95% Confidence intervals are shown for the differences of the means.

	Mean Levels After 12 weeks on Placebo (± SD)	**Mean Levels After 12 weeks on DHEA (± SD)**	**95% Confidence Intervals**	**p value**
Mitochondrial Protein Synthesis Rates (%/hr)	0.0569 (0.02)	0.053 (0.087)	-0.0228, 0.015	0.375
Sarcoplasmic Protein Synthesis Rates (%/hr)	0.0363 (0.009)	0.0378 (0.006)	-0.01, 0.013	0.813
Mixed Muscle Protein Synthesis Rates (%/hr)	0.057 (0.02)	0.0529 (0.009)	-0.023, 0.015	0.375

Skeletal Muscle Protein Synthesis Rates – Discussion

Very little work has previously been done looking at the effect of hypoadrenalism on skeletal muscle protein synthesis rates. Animal work in rats subjected to a hyperinsulinaemic euglycaemic clamp has shown that adrenalectomy per se does not affect skeletal muscle protein synthesis rates [272]. These authors showed that corticosteroid deficiency enhances the insulin sensitivity of muscle protein synthesis, and that restoring glucocorticoids blunts that increased sensitivity. These authors went on to shown that the mechanism by which this increased protein synthesis occurred was mediated by increased phosphorylation of translation initiation-regulatory proteins. These results are illustrated in Figure 19.

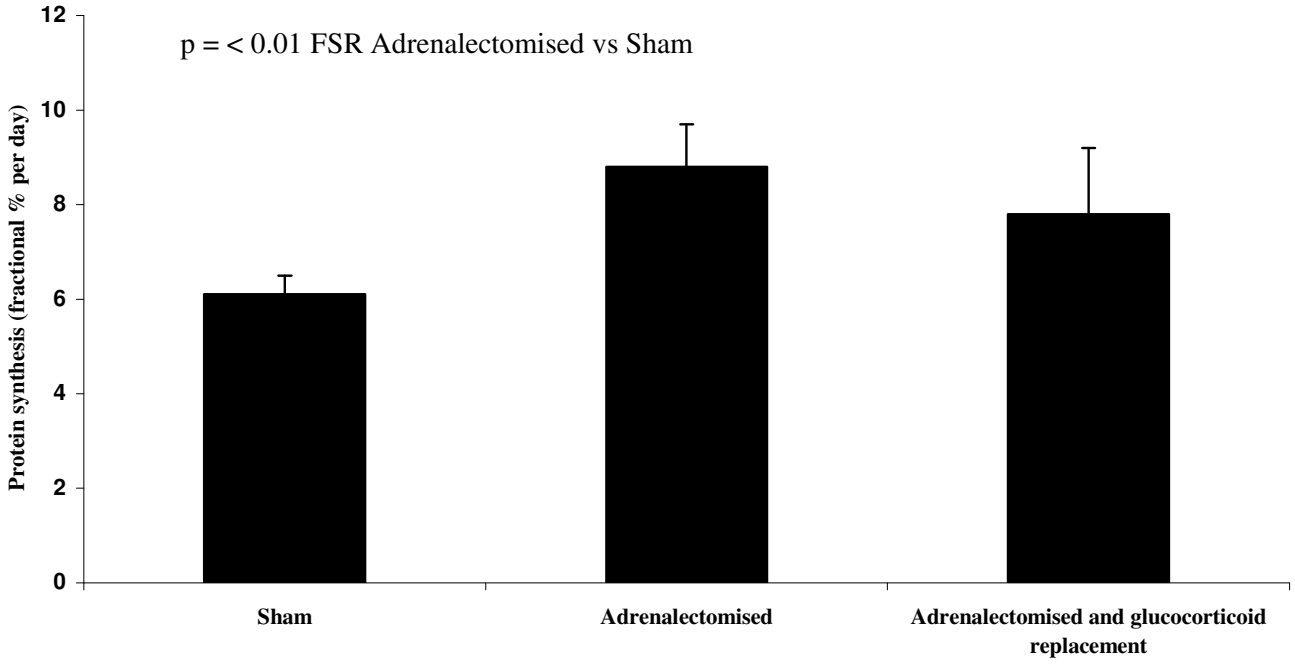


Figure 19 Fractional Synthesis Rates in Adrenalectomised Rats On and Off Glucocorticoids

As the subjects in the present study were on adequate doses of corticosteroids (as judged both clinically and biochemically), they would fall into the category on the far right of Figure 19. Thus, the enhancement of protein synthesis seen in the adrenalectomised animals was blunted by corticosteroid treatment. Clearly, it is impossible to test this hypothesis in humans, and whether the results from rats are applicable to humans is difficult due to the differences between species with respect to endogenous DHEA production.

Extrapolating these results to whole body may account for the lack of change in skeletal muscle protein synthesis rates – and possibly body composition, seen with DHEA. However, there are currently no data looking at the relationship between DHEA(S) levels and markers of skeletal muscle metabolism. Previous studies suggest that DHEA(S) administration increases lean mass, but the benefits versus risks of DHEA replacement on muscle mass and muscle strength in elderly individuals remain uncertain [60;253]. The present study also did not find a change in body composition, thus it is not surprising that there was a corresponding lack of change in the protein synthesis rate.

Mitochondrial Enzyme Activity – Results

In the 7 subjects in whom enzyme activities were measured, there were no changes during the DHEA arm of the study, when compared to placebo. These results are shown in Table 11.

Table 11 Mitochondrial Enzyme Activity

95% Confidence intervals are shown for the differences of the means.

	Mean Levels After 12 weeks on Placebo (± SD)	**Mean Levels After 12 weeks on DHEA (± SD)**	**95% Confidence Intervals**	**p value**
Cytochrome C Oxidase uU/g tissue	16.56 (3.86)	18.77 (6.21)	-1.85, 6.26	0.25
Cytochrome C Oxidase uU/g protein	89.22 (22.48)	96.35 (31.11)	-12.12, 26.38	0.547
Citrate Synthase uU/g tissue	25.44 (6.40)	27.49 (6.89)	-0.71, 4.81	0.25
Citrate Synthase uU/g protein	137.07 (36.63)	142.11 (35.66)	-7.42, 17.5	0.461

Mitochondrial Enzyme Activity – Discussion

These two enzymes were chosen as they form an integral part of the electron transport chain, important in ATP production. Cytochrome c oxidase is encoded by nuclear DNA, whilst citrate synthase is encoded by mitochondrial DNA, thus, the electron transport chain and ATP production is partly under the control of nuclear genes and partly mitochondrial.

Previous work has shown that in healthy individuals insulin infusion can increase mitochondrial mRNA expression and protein levels [273]. In the same study, these authors also went on to show that the response to insulin stimulated mitochondrial mRNA expression was blunted in subjects with type 2 diabetes. Thus, insulin and the cellular response to insulin plays a key role in maintaining mitochondrial ATP production. Mitochondrial enzyme activity can be used as a surrogate for ATP production rates.

There is currently no reported literature to assess the effects of hypoadrenalism on mitochondrial enzyme activity or ATP production. The present study has shown that in adequately treated non-diabetic hypoadrenal subjects, enzyme activity rates remain unchanged, thus suggesting that there is nothing blunting, or inhibiting the actions of these enzymes. These findings suggest that the improvement in insulin sensitivity seen in this study is not via this mechanism.

Alternatively, it can also be seen that there is no enhancement of enzyme activity, and thus there is unlikely to be any increased ATP availability. This may account for the lack of change in the physical performance seen either when assessing VO_2 max, or skeletal muscle strength.

The enzyme activities in our subjects compare favorably with those found in healthy volunteers. Enzyme activity in healthy subjects changes with age, with

enzyme activity decreasing over time. This decline can be attenuated by increasing insulin sensitivity, e.g. by increasing aerobic exercise [274]. Short et al studied 102 subjects aged between 21 and 87 years and subjected them to a 16-week protocol of incrementally graded aerobic exercise, or sham exercise (stretching). This study showed that in the exercise group there was an increase in mitochondrial enzyme activity, a decline in intramyocyte triglyceride level, and increased skeletal muscle GLUT 4 expression. These changes were most pronounced in the younger age group, with an increase in insulin sensitivity in this group.

Carryover Effects

Data was analysed to assess the effects of any potential carry over effects for those subjects given DHEA during the first phase of the study. There were no significant differences in any measured variable. For subjects given placebo in the first arm, there was a significant reduction in peak bike power in the DHEA arm of the study, (127.86 ± 36.67 vs 118 ± 32.94 watts, placebo vs DHEA, $p = 0.025$). There was, however, a small but statistically significant increase in biceps curl strength on DHEA when given during the second arm of the study, (80.67 ± 16.24 vs 84.33 ± 18.01 lbs, placebo vs DHEA, $p = 0.047$). It is likely that both of these measurements are Type 1 errors.

Summary and Conclusions

The major new finding of this study was that 50 mg of DHEA given for 3 months significantly improved insulin sensitivity in Caucasian hypoadrenal women aged between 20 and 80 years old. In addition there were significant reductions in total cholesterol, triglycerides and HDL cholesterol. These changes were achieved without a change in body composition.

In addition, there were no changes in measures of physical activity or exercise capacity. These were confirmed by biochemical assessments of mitochondrial enzyme activity, and fractional synthesis rates of mixed muscle protein, as well as mitochondrial and sarcoplasmic subfractions of skeletal muscle.

DHEA and DHEAS remain intriguing hormones. Their metabolites have a variety of effects in a number of physiological systems, and yet at present, little is known about the role of either DHEA or DHEAS in normal physiology. It has not yet been clearly identified if it is correct to classify ageing as a DHEA 'deficient' state. In hypoadrenal subjects, DHEA deficiency has been associated with a lower quality of life. It is clear, however, that these hormones are not essential for life, as adrenalectomised or hypoadrenal subjects who have little or no circulating DHEA do not have shortened life spans [275].

There has been tantalising evidence to support the use of DHEA in hypoadrenal subjects (Tables 3, 4, 7, and 8). The lay press has widely promoted the use of DHEA in normal healthy individuals, and bodybuilders promote its use as a method of increasing muscle mass. However, many of the claims made on the internet websites ('fountain of youth', 'prevents diabetes', 'prevents ageing', 'boosts the immune system', etc,) fail to mention that most of these studies were carried out either in vitro or in animals. These reports are further misleading as they also fail to

state that the results were usually a response to highly suprapharmacological doses of DHEA. There is also concern regarding the degree of quality control of the substances currently marketed. Finally, there remain valid concerns about the use of DHEA in individuals with a history of sex hormone dependent malignancies. Larger scale human studies are needed to answer many of these intriguing questions.

Assumptions, Limitations and Criticisms of the Present Study

The assumption for the current study was that the actual population that was sampled was representative of hypoadrenal women in general. The limitations of the actual sample were that it was limited to women either seen at Mayo Clinic in the 5 years previous to the start of recruitment (n = 23), or women who subscribed to internet self help groups who had never been previously seen at Mayo Clinic (n = 10). Thus there is an element of respondent or volunteer bias. This latter category assumed some degree of computer literacy and so may not have been representative of the hypoadrenal population in general, although this is possibly now less likely in North America. One advantage of internet recruitment is that the volunteers are not limited to subjects seen at a tertiary referral centre. The median distance travelled to the study centre was 286 miles (interquartile range = 59.5 to 358.5 miles). This wide geographical distribution of volunteers may decrease the generalisability of the results as it suggests that many of those seen at Mayo Clinic were tertiary referrals, and thus these results are open to referral bias. However, as a substantial minority of the volunteers in the study were not patients of Mayo Clinic, it is likely that the results of the current study may be more applicable to a wider population. It is accepted, however, that these results are only directly applicable to Caucasian women with hypoadrenalism due to Addison's disease or bilateral adrenalectomy. Whether these results can be applied to other categories of hypoadrenal subjects, e.g. men or non-Caucasians, remains to be determined. It is also possible that those hypoadrenal women seen at Mayo in the 5 years prior to the start of the study who did not respond to the first two sets of invitation letters to participate in the study were in some way systematically different to those who did respond. A similar bias may have been present when considering those women who answered the internet advertisement.

There was no method to minimise or evaluate this potential source of bias in this study. Whether a difference in adrenal hormonal status makes a difference to motivation is unknown.

During the recruitment phase of the study the Women's Health Initiative (WHI) was published, which suggested that postmenopausal women who had a uterus and were on combined oestrogens and progestins were at a slightly increased risk of developing cancers of the breast and bowel [276]. One of the inclusion criteria for the study was that subjects had steady oestrogen status for 6 months prior to entering the study. Publication of the WHI results made this very difficult and so recruitment included subjects who may have stopped oestrogens only a few weeks prior to joining the study.

Two volunteers had previously been on DHEA at various doses. Both had stopped at least 6 months prior to starting the study, however, it is possible that these women had a 'memory' effect and knew what effects to look for when on the DHEA.

In previous studies looking at muscle protein synthesis it has been important to have a weight maintaining diet for at least three days prior to the muscle biopsy. In the present study however, due to logistical problems involved with the subjects travelling long distances, it was felt that this would not be possible. To ensure consistency however, subjects were given either standardised meals for the three days prior to biopsy, or given comprehensive instructions as to the diet they should follow. This was done by the experienced research centre dieticians. Whatever policy was given for the first visit was followed for the second visit.

Hypoadrenal women have been shown to have virtually no circulating androgens 5 years after the menopause [277]. The validity of the findings of the present

study would have been increased had we recruited only postmenopausal, hypoadrenal women, as the ovaries would not have been producing any endogenous androgens.

For the statistical assumptions it is clear that subjects were not randomly selected as they were mainly from the Mayo Clinic records. However, this study included a wide range of ages and the results should therefore be applicable to 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 to be determined.

Strength testing and exercise are related to motivation [278]. Subjects who feel lower in mood may not be motivated to perform to their maximum ability and so the results may be misleading. However, if DHEA(S) improved mood, then the resulting increase in exercise capacity may be overestimated because of this. Data presented elsewhere showed that there was a very modest improvement in mood [79], but this improvement did not translate into an increase in exercise capacity.

Limitations of Muscle Mass Measurements

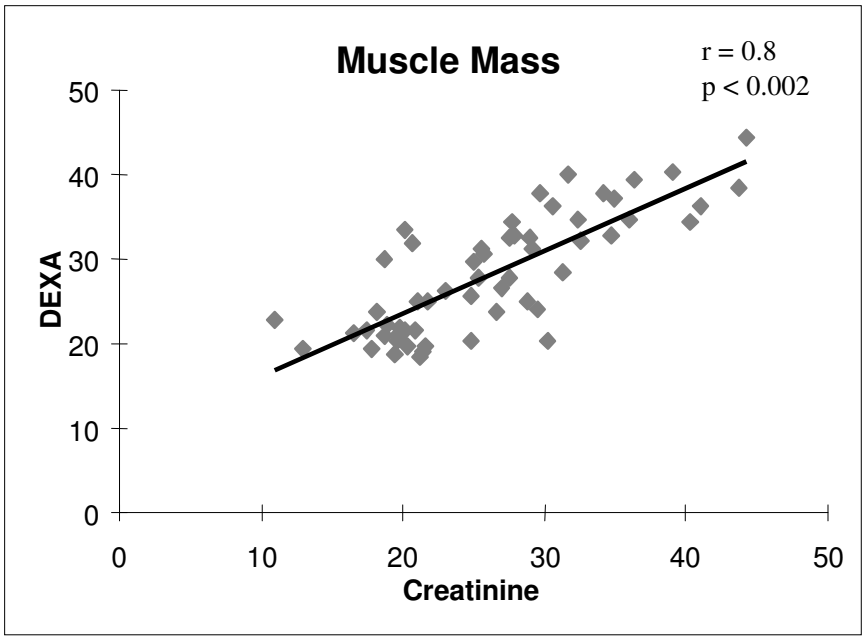
Doubts have been cast about the sensitivity of DEXA to measure changes in muscle mass in older people undergoing an exercise program [279]. Nair et al have previously found excellent correlation between muscle mass estimated from DEXA and creatinine excretion in a group of 58 subjects of which 24 were age 65 to 94 years, 24 were 45 to 58 years, and 10 were 20 to 30 years ($r = 0.8$, $P < 0.002$) as shown in Figure 20. DEXA measures non-skeletal FFM and fat mass but not muscle mass. DEXA (Lunar DPX-L, Madison, WI) measures FFM in four limbs. Non-skeletal FFM is almost exclusively muscle and whole body muscle mass can be estimated based on the assumption that 75% of the muscle mass is located in the four extremities [280]. Although not a direct measurement of muscle mass, it is a technique easily done and highly reproducible (coefficient of variation < 4%). The appendicular skeletal muscle mass was most relevant in the present study because leg muscles were biopsied for protein synthesis measurements. We did not anticipate any problems in using DEXA for cross sectional comparison because of its high reproducibility and excellent correlation to creatinine excretion. In addition total body water measurements would have allowed the determination of any differences in water retention between groups, which could not be measured using DEXA. As no changes in body composition were demonstrated by DEXA in the present study, the samples were not analysed for the total body water data.

Limitations of Testing Muscle Strength and Peak VO_2 Max

There are numerous tests to assess muscle functions in humans. In the current study subjects were asked to carry out a limited number of exercises because it was felt that they should be subjected to only a minimum number of cost-effective tests. The proposed procedures to test muscle function were well validated at Mayo Clinic and were extensively used by many investigators, permitting a direct comparison of data from the current study with that of others. Because of the need for test specificity, 1RM evaluations were used to provide the most direct evaluation of the gains made over a specific period of time from DHEA(S) replacement.

The isometric and isokinetic tests focused on the quadriceps muscle group because it was the vastus lateralis muscle from which needle biopsy samples were taken and it is the muscle most critical for activities of daily living such as standing, walking and climbing stairs.

Figure 20 Correlation Between Skeletal Muscle Mass, DEXA and Urinary Creatinine Excretion



Adverse Effects and Potential Limitations of DHEA Use

Adverse effects were common in most previous studies, and were minor in nature. These were usually in women and were due to the androgenic effects of the DHEA metabolites. As in the present study, the most common adverse effect was an increased skin sebum production leading to perceived 'greasiness' and acne [71]. However, many women have previously reported that this change is beneficial and that prior to DHEA replacement their skin was excessively dry. This effect has been reversible when the DHEA was withdrawn.

Of greater concern, are the reports of mild elevations in serum transaminases. These occur within a few weeks of starting the DHEA. As with our one case of raised ALT, these increases have not led to any cases of subjects having to withdraw from a study. In addition, effects have either been reversible when the drug has been stopped, or have become normal after a few weeks on the drug [21]. Changes in haematological values and liver function tests for the present study are shown in Table 12.

Nonsignificant rises in haemoglobin and haematocrit have been reported in some trials, possibly due to the androgenic effects of DHEA metabolites [224]. No evidence of these changes occurred in the present study.

Other, milder adverse effects reported by others included increases in perspiration and body odour [74;80;224]. The results from the present study are in agreement with several others, showing an increase in body hair – especially facial, axillary and pubic hair [21;22;224]. Rarely, hair loss has also been reported [21].

Other side effects include abdominal pain, intermenstrual bleeding, fatigue, insomnia, skin rash, weight gain, and breast tenderness [71;281]. These side effects occurred at higher doses of DHEA (100 mg and 200 mg). All of these effects were

transitory and were reversible on withdrawal of the drug. None of these side effects were observed in the present study.

There are more serious potential risks with the use of DHEA. While there are several rodent models showing that the use of DHEA prevents tumourigenesis [282], the use of suprapharmacological doses of DHEA also has resulted in an increase in hepatocellular carcinoma [283]. Because of the conversion of DHEA into androgens and oestrogens, use of supplemental DHEA in individuals with a history of sex hormone dependent malignancy such as prostate, breast or endometrial cancer remains a valid concern. This issue has yet to be fully addressed in long term human studies.

Table 12 Haematological Values and Liver Function Tests

95% Confidence intervals are shown for the differences of the means

	Mean Levels After 12 weeks on Placebo (± SD)	**Mean Levels After 12 weeks on DHEA (± SD)**	**95% Confidence Intervals**	**p value**
Haemoglobin (g/dl)	13.1 (0.8)	13.2 (1.1)	-0.2, 0.4	0.511
Haematocrit (%)	38.01 (2.56)	38.46 (3.0)	-0.31, 1.20	0.195
Aspartate Transaminase (U/l)	24.71 (9.99)	23.21 (7.83)	-4.14, 1.14	0.174
Alanine Transaminase (U/l)	23.29 (23.71)	21.57 (11.64)	-9.93, 6.50	0.824
Alkaline Phosphatase (U/l)	145.0 (72.8)	130.8 (53.2)	-46.3, 18.0	0.579

Availability of DHEA Within the United States

DHEA in dietary supplements is synthetic, manufactured from plant chemicals found in soybeans and wild yam. DHEA has been banned by the International Olympic Committee because of its conversion to sex hormones, and so potentially be used as a drug of abuse. In the USA, the Food and Drug Administration also banned the substance until the passage of the Dietary and Supplement Health and Education Act of 1994, when this ruling was overturned. DHEA is now freely available in pharmacies and health food shops, where it is classed as a food supplement. 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. It can be sold directly to the public as long as no claims are made about therapeutic efficacy. In the USA food supplements are not required to undergo strict safety and efficacy testing and as a result in these over-the-counter products, there are problems with quality control [284]. This study by Parasrampuria et al showed that the quantity of DHEA from different manufacturers in a variety of different doses, varied from 0% to 150% of what the label claimed was in the product. One further private company has reported quality control on commercially available DHEA (http://www.consumerlab.com/results/dhea.asp). This company found that 3 of the 17 products tested by them contained significantly less DHEA than claimed. One product boasting "Pharmaceutical Quality" and "...produced and packaged in [an] OTC approved facility" was found to have only 19% of the claimed DHEA. Another product was found to have only 79% of the claimed DHEA. Another product indicated that its raw material met United States Pharmacopoeia standards. The supply of DHEA for the present study was pharmaceutical grade and manufactured specifically for the study, however, once the study was completed, those subjects who

felt better on the DHEA who wanted to continue on it, were advised to purchase it commercially (General Nutrition Companies, Inc., Pittsburgh, PA). They were, however, warned of the findings quoted above.

In an attempt to reduce the potential abuse of DHEA, a bill has recently been put before the US House of Representatives that aims to restrict the over-the-counter sale of DHEA and other androgenic steroid precursors [285]. How this will affect those people who may derive benefit from DHEA, such as hypoadrenal and elderly subjects, remains to be determined.

Potential Future Studies

The present study could be repeated with only hypoadrenal women who were at least 5 years past the menopause to ensure that any circulating androgens present during the study would be due to administered DHEA(S) only.

The results showing the increased insulin sensitivity could be followed up by studying the effects of DHEA on insulin sensitivity in subjects with diabetes. In addition, it could be used in situations where hyperglycaemia occurs in addition to relative hypoadrenalism, e.g. acute critical illness, to see if outcomes are altered.

The effect of DHEA(S) replacement could be investigated in hypoadrenal men. This would allow the determination of the relative contribution made to circulating androgens by the testis, and from adrenal precursors from the adrenal glands.

In vivo and in vitro studies of mitochondrial function studies in a variety of tissues with DHEA(S) replacement could be undertaken to assess prevention of radical oxygen species damage. This would assume that DHEA(S) reduces free radicals, and would help to clarify whether the free radical theory of ageing was correct [109].

Further work could be done looking at the mechanisms of the DHEA induced increase in insulin sensitivity. As the present study showed that this was due to an increase in peripheral glucose uptake, particular efforts on the role of skeletal muscle glucose oxidation and muscle bioenergitics might be an initial focus. These may include measurements of intramyocellular and intrahepatic lipid levels using recently described proton magnetic spectroscopic methods [286].

Longer term studies of DHEA(S) replacement, looking at markers of cardiovascular disease, such as C - Reactive Protein, and relating these findings to

cardiovascular outcomes such as fatal and non fatal myocardial infarction and stroke could be done. Whilst there are longitudinal epidemiological studies looking at DHEA levels and cardiovascular outcomes, there are no interventional randomised studies. This would require a large financial investment, and is unlikely to be carried out.

Animal studies involving rabbits fed either a normal or a highly atherogenic diet each with or without DHEA supplementation showed that whilst the DHEA supplementation of the highly atherogenic diet did not decrease the elevated lipid peroxidase levels, it did reverse the lowered superoxide dismutase activity seen in the unsuppplemented diet [287]. DHEA also increased superoxide dismutase activity in the animals fed with a normal diet. Superoxide dismutase is one of the main enzymes involved in protecting against free radical damage. This effect needs to be validated in human studies.

IGF 2 rises quickly with intense exercise in young men [288] with IGF 1 and IGF 2 peaks being achieved before the GH peak. It is not known what the DHEA levels were after short-term acute exercise. An additional study would be to assess the correlation between IGF 2 rise and DHEA levels.

In August 2002 Genelabs Inc in California were granted FDA approval for a New Drug Application on a compound (Prestara™) that contains prasterone, a synthetic form of DHEA as its active ingredient. They had previously carried out several studies looking at the effects of DHEA in patients with systemic lupus erythematosus that had shown beneficial effects in this condition [71;289;290]. This, or similar products could assess effects of DHEA(S) in other conditions.

Publications Resulting From This Research

Dhatariya K, Nair KS. Dehydroepiandrosterone – is there a role for replacement? Mayo Clinic Proceedings 2003;78(10):1257-1273.

Dhatariya K. Is there a role for dehydroepiandrosterone replacement in the intensive care population? Intensive Care Medicine 2003;29(11):1877-1880.

Dhatariya K, Bigelow ML, Nair KS. The effects of dehydroepiandrosterone replacement on insulin sensitivity and lipids in hypoadrenal women. (Submitted for publication).

Dhatariya K, Smith GE, Nair KS. The effect of dehydroepiandrosterone replacement on mood, memory, well-being and sexual function in hypoadrenal women. (Submitted for publication).

Dhatariya K, Bigelow ML, Nair KS. The effect of dehydroepiandrosterone replacement on muscle strength and physiology in hypoadrenal women. (Submitted for publication).

Reference List

1. Roth GS, Lane MA, Ingram DK, Mattison JA, Elahi D, Tobin JD *et al*. Biomarkers of caloric restriction may predict longevity in humans. *Science* 2002;**297**:811 .

2. Kolata, G. A therapy to restore body, pep of youth? Expensive hormone procedure has both supporters, critics. The San Diego Union-Tribune , A3-A24. 22-12-2002. San Diego, California.

3. Vesalius A. De Humani Corporis Fabrica. Basileae: Ex officina Joannis Poprini, 1543.

4. Eustachius B. Opuscula Anatomica de Renum Structura, Efficio et Administratione. Venice: Vicentius Luchinus, 1564.

5. Addison T. On the constitutional and local effects of disease of the supra-renal capsules. *Medical Classics* 1937;**2**:244-80.

6. Sorkin SZ. A centenary of Addison's disease. *Bull.NY.Acad.Med.* 1956;**32**:811-36.

7. Brown-Sequard E. Recherches experimentales sur le physiologie et la pathologie des capsules surrenales. *Arch.Gen.de Med.* 1856;**8**:385-401.

8. Takamini J. The blood-pressure raising principle of the suprarenal glands. *Therapeutic Gazette* 1901;**17**:224.

9. Pfiffner JJ, Swingle WW. The preparation of an active extract of the suprarenal cortex. *Anat.Rec.* 1929;**94**:225.

10. Steiger M, Reichenstein T. Partial synthesis of a crystalized compound with the biological activity of the adrenal cortical hormone. *Nature* 1937;**139**:825-6.

11. Arlt W, Allolio B. Adrenal insufficiency. *The Lancet* 2003;**361**:1881-93.

12. Ten S, New M, Maclaren N. Addison's disease 2001. *J Clin Endocrinol Metab* 2001;**86** :2909-22.

13. Ronghe MD, Barton J, Jardine PE, Crowne EC, Webster MH, Armitage M *et al*. The importance of testing for adrenoleucodystrophy in males with idiopathic Addison's disease. *Arch.Dis.Child.* 2002;**86**:185-9.

14. Rao RH, Vagnucci AH, Amico JA. Bilateral massive adrenal hemorrhage: early recognition and treatment. *Ann.Intern.Med.* 1989;**110**:227-35.

15. Lam KY, Lo CY. Metastatic tumours of the adrenal glands: a 30-year experience in a teaching hospital. *Clin.Endocrinol.(Oxf)* 2002;**56**:95-101.

16. Dobs AS, Dempsey MA, Ladenson PW, Polk BF. Endocrine disorders in men infected with human immunodeficiency virus. *Am.J.Med.* 1988;**84**:611-6.

17. Kumar N, Singh S, Govil S. Adrenal histoplasmosis: clinical presentation and imaging features in nine cases. *Abdom.Imaging* 2003;**28**:703-8.

18. Glasgow BJ, Steinsapir KD, Anders K, Layfield LJ. Adrenal pathology in the acquired immune deficiency syndrome. *Am.J.Clin.Pathol.* 1985;**84**:594-7.

19. Grinspoon SK, Bilezikian JP. HIV disease and the endocrine system. *N.Eng.J.Med.* 1992;**327**:1360-5.

20. Watson JP, Lewis RA. Schmidt's syndrome associated with sarcoidosis. *Postgrad.Med.J.* 1996;**72**:435-6.

21. Arlt W, Callies F, van Vlijmen JC, Koehler I, Reincke M, Bidlingmaier M *et al*. Dehydroepiandrosterone replacement in women with adrenal insufficiency. *N.Eng.J.Med.* 1999;**341**:1013-20.

22. Hunt PJ, Gurnell EM, Huppert FA, Richards C, Prevost AT, Wass JA *et al*. Improvement in mood and fatigue after dehydroepiandrosterone replacement in Addison's disease in a randomized, double blind trial. *J.Clin.Endocrinol.Metab.* 2000;**85**:4650-6.

23. Løvås K, Loge JH, Husebye ES. Subjective health status in Norwegian patients with Addisons disease. *Clin.Endocrinol.(Oxf)* 2002;**56**:581-8.

24. Nieschlag E, Loriaux DL, Ruder HJ, Zucker IR, Kirschner MA, Lipsett MB. The secretion of dehydroepiandrosterone and dehydroepiandrosterone sulphate in man. *J.Endocrinol.* 1973;**57**:123-34.

25. Liu CH, Laughlin GA, Fischer UG, Yen SS. Marked attenuation of ultradian and circadian rhythms of dehydroepiandrosterone in postmenopausal women: evidence for a reduced 17,20-desmolase enzymatic activity. *J.Clin.Endocrinol.Metab.* 1990;**71**:900-6.

26. Allolio B, Arlt W. DHEA treatment: myth or reality? *Trends Endocrinol.Metab.* 2002;**13**:288-94.

27. Butenandt A, Dannenbaum H. Isolierung eines neuen, physiologisch unwirksamen Sterinderivates aus Mannerharn, seine Verknupfung mit Dehydro-androsteron und Androsterone: ein Bietrag zur Konstitution des Androsterons. *Z.Physiol.Chem.* 1934;**229**:192-208.

28. Migeon CJ, Plager JE. Identification and isolation of dehydroisoandrosterone from peripheral human plasma. *J.Biol.Chem.* 1954;**209**:767-72.

29. Baulieu EE. Three sulfate esters of 17-ketosteroids in the plasma of human subjects before and after the administration of ACTH. *J.Clin.Endocrinol.* 1960;**20**:900-4.

30. Jefcoate C. High-flux mitochondrial cholesterol trafficking, a specialized function of the adrenal cortex. *J.Clin.Invest.* 2002;**110**:881-90.

31. Labrie F. Intracrinology. *Mol.Cell.Endocrinol.* 1991;**78**:C113-8.

32. Baulieu EE, Robel P. Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) as neuroactive neurosteroids. *Proc.Natl.Acad.Sci.USA* 1998;**95**:4089-91.

33. Rosenfeld RS, Rosenberg BJ, Fukushima DK, Hellman L. 24-Hour secretory pattern of dehydroisoandrosterone and dehydroisoandrosterone sulfate. *J.Clin.Endocrinol.Metab.* 1975;**40**:850-5.

34. Labrie F, Belanger A, Cusan L, Gomez JL, Candas B. Marked decline in serum concentrations of adrenal C_{19} sex steroid precursors and conjugated androgen metabolites during aging. *J.Clin.Endocrinol.Metab.* 1997;**82**:2396-402.

35. Frye RF, Kroboth PD, Kroboth FJ, Stone RA, Folan M, Salek FS *et al*. Sex differences in the pharmacokinetics of dehydroepiandrosterone (DHEA) after single- and multiple-dose administration in healthy older adults. *J.Clin.Pharmacol.* 2000;**40**:596-605.

36. Liu D, Dillon JS. Dehydroepiandrosterone activates endothelial cell nitric-oxide synthase by a specific plasma membrane receptor coupled to $G_{i2,3}$. *J.Biol.Chem.* 2002;**277**:21379-88.

37. Meikle AW, Dorchuck RW, Araneo BA, Stringham JD, Evans TG, Spruance SL *et al*. The presence of a dehydroepiandrosterone-specific receptor binding complex in murine T cells. *J.Steroid Biochem.Mol.Biol.* 1992;**42**:293-304.

38. Meikle AW, Daynes RA, Araneo BA. Adrenal androgen secretion and biologic effects. *Endocrinol.Metab.Clin.North Am.* 1991;**20**:381-400.

39. Labrie F, Luu-The V, Lin SX, Simard J, Labrie C. Role of 17 beta-hydroxysteroid dehydrogenases in sex steroid formation in peripheral intracrine tissues. *Trends Endocrinol.Metab.* 2000;**11**:421-7.

40. Orentreich N, Brind JL, Rizer RL, Vogelman JH. Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. *J.Clin.Endocrinol.Metab.* 1984;**59**:551-5.

41. Birkenhager-Gillesse EG, Derksen J, Lagaay AM. Dehydroepiandrosterone sulphate (DHEAS) in the oldest old, aged 85 and over. *Ann.NY.Acad.Sci.* 1994;**719**:543-52.

42. Hornsby PJ. Aging of the human adrenal cortex. *Ageing Res.Rev.* 2002;**1**:229-42.

43. Tsagarakis S, Grossman A. The hypothalamic-pituitary-adrenal axis in senescence. In Morley JE, Korenman SG, eds. *Endocrinology and metabolism in the elderly*, pp 70-91. London: Blackwell Scientific Publications, 1992.

44. Nawata H, Yanase T, Goto K, Okabe T, Ashida K. Mechanism of action of anti-aging DHEA-S and the replacement of DHEA-S. *Mech.Ageing Dev.* 2002;**123**:1101-6.

45. Ohashi M, Kato K, Nawata H, Ibayashi H. Adrenocortical responsiveness to graded ACTH infusions in normal young and elderly human subjects. *Gerontology* 1986;**32**:43-51.

46. Salek FS, Bigos KL, Kroboth PD. The influence of hormones and pharmaceutical agents on DHEA and DHEA-S concentrations: a review of clinical studies. *J.Clin.Pharmacol.* 2002;**42**:247-66.

47. Lasley BL, Santoro N, Randolf JF, Gold EB, Crawford S, Weiss G *et al*. The relationship of circulating dehydroepiandrosterone, testosterone, and estradiol to stages of the menopausal transition and ethnicity. *J.Clin.Endocrinol.Metab.* 2002;**87**:3760-7.

48. Tsuji K, Furutama D, Tagami M, Ohsawa N. Specific binding and effects of dehydroepiandrosterone sulfate (DHEA-S) on skeletal muscle cells: possible implication for DHEA-S replacement therapy in patients with myotonic dystrophy. *Life Sci.* 1999;**65**:17-26.

49. Simoncini T, Mannella P, Fornari L, Varone G, Caruso A, Genazzani AR. Dehydroepiandrosterone modulates endothelial nitric oxide synthesis via direct genomic and nongenomic mechanisms. *Endocrinology* 2003;**144**:3449-55.

50. Suzuki T, Suzuki N, Daynes RA, Engleman EG. Dehydroepiandrosterone enhances IL2 production and cytotoxic effector function of human T cells. *Clin.Immunol.Immunopathol.* 1991;**61**:202-11.

51. Labrie F, Dupont A, Belanger A. Important advances in oncology. Philadelphia: JB Lippencott, 1985.

52. Cumming DC, Rebar RW, Hopper BR, Yen SS. Evidence for an influence of the ovary on circulating dehydroepiandrosterone sulfate levels. *J.Clin.Endocrinol.Metab.* 1982;**54**:1069-71.

53. Vermeulen A. The hormonal activity of the postmenopausal ovary. *J.Clin.Endocrinol.Metab.* 1976;**42**:247-53.

54. Judd HL, Judd GE, Lucas WE, Yen SS. Endocrine function of the postmenopausal ovary: concentration of androgens and estrogens in ovarian and peripheral vein blood. *J.Clin.Endocrinol.Metab.* 1974;**39**:1020-4.

55. Judd HL, Lucas WE, Yen SS. Effect of oophorectomy on circulating testosterone and androstenedione levels in patients with endometrial cancer. *Am.J.Obstet.Gynecol.* 1974;**118**:793-8.

56. Legrain S, Massien C, Lahlou N, Roger M, Debuire B, Diquet B *et al*. Dehydroepiandrosterone replacement administration: pharmacokinetic and

pharmacodynamic studies in healthy elderly subjects. *J.Clin.Endocrinol.Metab.* 2000;**85**:3208-17.

57. Young J, Couzinet B, Nahoul K, Chanson P, Baulieu EE, Schaison G. Panhypopituitarism as a model to study the metabolism of dehydroepiandrosterone (DHEA) in humans. *J.Clin.Endocrinol.Metab.* 1997;**82**:2578-85.

58. Morales AJ, Nolan JJ, Nelson JC, Yen SS. Effects of replacement dose of dehydroepiandrosterone in men and women of advancing age. *J.Clin.Endocrinol.Metab.* 1994;**78**:1360-7.

59. Arlt W, Callies F, Oettel M, Ernst M, Schulte HM, Allolio B. Oral dehydroepiandrosterone for adrenal androgen replacement: pharmacokinetics and peripheral conversion to androgens and estrogens in young healthy females after dexamethasone suppression. *J.Clin.Endocrinol.Metab.* 1998;**83**:1928-34.

60. Yen SS, Morales AJ, Khorram O. Replacement of DHEA in aging men and women. Potential remedial effects. *Ann.NY.Acad.Sci.* 1995;**774**:128-42.

61. Coleman DL, Leiter EH, Schwizer RW. Therapeutic effects of dehydroepiandrosterone (DHEA) in diabetic mice. *Diabetes* 1982;**31**:830-3.

62. Hansen PA, Han DH, Nolte LA, Chen M, Holloszy JO. DHEA protects against visceral obesity and muscle insulin resistance in rats fed a high-fat diet. *Am.J.Physiol.Regul.Integr.Comp.Physiol.* 1997;**273**:R1704-8.

63. Barrett-Connor E, Khaw KT, Yen SS. A prospective study of dehydroepiandrosterone sulfate, mortality, and cardiovascular disease. *N.Eng.J.Med.* 1986;**315**:1519-24.

64. Barrett-Connor E, Khaw KT. Absence of an inverse relation of dehydroepiandrosterone sulfate with cardiovascular mortality in postmenopausal women. *N.Eng.J.Med.* 1987;**317**:711.

65. Thoman ML, Weigle WO. The cellular and subcellular bases of immunosenescence. *Adv.Immunol.* 1989; **46**:221-61.

66. Helzlsouer KJ, Gordon GB, Alberg AJ, Bush TL, Comstock GW. Relationship of prediagnostic serum levels of dehydroepiandrosterone and dehydroepiandrosterone sulfate to the risk of developing premenopausal breast cancer. *Cancer Res.* 1992;**52**:1-4.

67. Gordon GB, Bush DE, Weisman HF. Reduction of atherosclerosis by administration of dehydroepiandrosterone. A study in the hypercholesterolemic New Zealand white rabbit with aortic intimal injury. *J.Clin.Invest.* 1988;**82**:712-20.

68. Arad Y, Badimon JJ, Badimon L, Hembree WC, Ginsberg HN. Dehydroepiandrosterone feeding prevents aortic fatty streak formation and

cholesterol accumulation in cholesterol-fed rabbit. *Arteriosclerosis* 1989;**9**:159-66.

69. Jesse RL, Loesser K, Eich DM, Qian YZ, Hess ML, Nestler JE. Dehydroepiandrosterone inhibits human platelet aggregation in vitro and in vivo. *Ann.NY.Acad.Sci.* 1995;**774**:271-90.

70. Hak AE, Witteman JC, de Jong FH, Geerlings MI, Hofman A, Pols HA. Low levels of endogenous androgens increase the risk of atherosclerosis in elderly men: the Rotterdam study. *J.Clin.Endocrinol.Metab.* 2002;**87**:3632-9.

71. Petri MA, Lahita RG, Van Vollenhoven RF, Merrill JT, Schiff M, Ginzler EM *et al*. Effects of prasterone on corticosteroid requirements of women with systemic lupus erythematosus: A double-blind, randomized, placebo-controlled trial. *Arthritis.Rheum.* 2002;**46**:1820-9.

72. Valenti, G, Maggio, M, Ceresini, G, Denti, L, Banchini, A, Corsi, A M, and Ferrucci, L. The relationship between DHEAS levels and muscle strength in men: Results from the InCHIANTI study. Abstract presented at The Endocrine Society Meeting, San Francisco P3 - 320. 2002.
Ref Type: Abstract

73. Short KR, Nair KS. Mechanisms of sarcopenia of aging. *J.Endocrinol.Invest.* 1999;**22**:95-105.

74. Gebre-Medhin G, Husebye ES, Mallmin H, Helstrom L, Berne C, Karlsson FA *et al*. Oral dehydroepiandrosterone (DHEA) replacement therapy in women with Addison's disease. *Clin.Endocrinol.(Oxf)* 2000;**52**:775-80.

75. Callies F, Koehler I, Seibel MJ, Arlt W, Allolio B, Fassnacht M *et al*. Dehydroepiandrosterone replacement in women with adrenal insufficiency: effects on body composition, serum leptin, bone turnover, and exercise capacity. *J.Clin.Endocrinol.Metab.* 2001;**86**:1968-72.

76. Oelkers W. Adrenal insufficiency. *N.Eng.J.Med.* 1996;**335**:1206-12.

77. Riedel M, Wiese A, Schurmeyer TH, Brabant G. Quality of life in patients with Addison's disease: effects of different cortisol replacement modes. *Exp.Clin.Endocrinol.* 1993;**101**:106-11.

78. Arlt W. Quality of life in Addisons disease - the case for DHEA replacement. *Clin.Endocrinol.(Oxf)* 2002;**56**:573-4.

79. Dhatariya, K. Assessing the effects of dehydroepiandrosterone (DHEA) replacement on the mood and well-being of hypoadrenal women (Masters Thesis, Mayo Graduate School). 2004. Mayo Graduate School.

80. Løvås K, Gebre-Medhin G, Fougner KJ, Uhlving S, Nedrebø BG, Myking OL *et al*. Replacement of dehydroepiandrosterone in adrenal failure: no benefit for subjective health status and sexuality in a 9-month, randomized, parallel group clinical trial. *J.Clin.Endocrinol.Metab.* 2003;**88**:1112-8.

81. Cleary MP, Zisk JF. Anti-obesity effect of two different levels of dehydroepiandrosterone in lean and obese middle-aged female Zucker rats. *Int.J.Obes.Relat.Metab.Disord.* 1986; **10**:193-204.

82. Gansler TS, Muller S, Cleary MP. Chronic administration of dehydroepiandrosterone reduces pancreatic beta-cell hyperplasia and hyperinsulinemia in genetically obese Zucker rats. *Proc.Soc.Exp.Biol.Med.* 1985;**180**:155-62.

83. Han DH, Hansen PA, Chen MM, Holloszy JO. DHEA treatment reduces fat accumulation and protects against insulin resistance in male rats. *J.Gerontol.A Biol.Sci.Med.Sci.* 1998;**53**:B19-24.

84. Shepherd A, Cleary MP. Metabolic alterations after dehydroepiandrosterone treatment in Zucker rats. *Am.J.Physiol.Endocrinol.Metab.* 1984;**246**:E123-8.

85. Cleary MP, Zabel T, Sartin JL. Effects of short-term dehydroepiandrosterone treatment on serum and pancreatic insulin in Zucker rats. *J.Nutr.* 1988;**118**:382-7.

86. Bartness TJ, Billington CJ, Levine AS, Morley JE, Rowland NE, Brown DM. Insulin and metabolic efficiency in rats. II. Effects of NE and cold exposure. *Am.J.Physiol.Regul.Integr.Comp.Physiol.* 1986;**251**:R1118-25.

87. Taniguchi S, Yanase T, Haji M, Ishibashi K, Takayanagi R, Nawata H. The antiobesity effect of dehydroepiandrosterone in castrated or noncastrated obese Zucker male rats. *Obes.Res.* 1995;**3**:639S-43S.

88. Muller S, Cleary MP. Glucose metabolism in isolated adipocytes from lean and obese Zucker rats following treatment with dehydroepiandrosterone. *Metabolism* 1985;**34**:278-84.

89. Aoki K, Kikuchi T, Mukasa K, Ito S, Nakajima A, Satoh S. Dehydroepiandrosterone suppresses elevated hepatic glucose - 6 - phosphate mRNA level in C57BL/KsJ - db/db mice: comparison with troglitazone. *Endocr.J.* 2000;**47**:799-804.

90. Schriock ED, Buffington CK, Givens JR, Buster JE. Enhanced post-receptor insulin effects in women following dehydroepiandrosterone infusion. *J.Soc.Gynecol.Investig.* 1994;**1**:74-8.

91. Buffington CK, Givens JR, Kitabchi AE. Sensitivity of pyruvate dehydrogenase to insulin in activated T lymphocytes. Lack of responsiveness to insulin in patients with polycystic ovarian disease and diabetes. *Diabetes* 1990;**39**:361-8.

92. Wright BE, Browne ES, Svec F, Porter JR. Divergent effect of dehydroepiandrosterone on energy intakes of Zucker rats. *Physiol.Behav.* 1993;**53**:39-43.

93. Cleary MP, Shepherd A, Jenks B. Effect of dehydroepiandrosterone on growth in lean and obese Zucker rats. *J.Nutr.* 1984;**114**:1242-51.

94. Kajita K, Ishizuka T, Mune T, Miura A, Ishizawa M, Kanoh Y *et al*. Dehydroepiandrosterone down-regulates the expression of peroxisome proliferator-activated receptor γ in adipocytes. *Endocrinology* 2003;**144**:253-9.

95. Yorek MA, Coppey LJ, Gellett JS, Davidson EP, Bing X, Lund DD *et al*. Effect of treatment of diabetic rats with dehydroepiandrosterone on vascular and neural function. *Am.J.Physiol.Endocrinol.Metab.* 2002;**283**:E1067-E1075.

96. Ayhan S, Markal N, Siemionow K, Araneo B, Siemionow M. Effect of subepineurial dehydroepiandrosterone treatment on healing of transected nerves repaired with the epineurial sleeve technique. *Microsurgery* 2003;**23**:49-55.

97. Nair KS. Muscle protein turnover: methodological issues and the effect of aging. *J.Gerontol.A Biol.Sci.Med.Sci.* 1995;**50**:107-12.

98. Waterlow JC. Protein turnover with special reference to man. *Q.J.Exp.Physiol.* 1984;**69**:409-38.

99. Young A, Stokes M, Crowe M. The size and strength of the quadriceps muscles of old and young men. *Clin.Physiol.* 1985;**5**:145-54.

100. Yarasheski KE, Zachwieja JJ, Bier DM. Acute effects of resistance exercise on muscle protein synthesis rate in young and elderly men and women. *Am.J.Physiol.Endocrinol.Metab.* 1993;**265**:E210-4.

101. Welle S, Thornton C, Jozefowicz R, Statt M. Myofibrillar protein synthesis in young and old men. *Am.J.Physiol.Endocrinol.Metab.* 1993;**264**:E693-8.

102. Yarasheski KE, Campbell JA, Smith K, Rennie MJ, Holloszy JO, Bier DM. Effect of growth hormone and resistance exercise on muscle growth in young men. *Am.J.Physiol.Endocrinol.Metab.* 1992;**262**:E261-7.

103. Balagopal P, Rooyackers OE, Adey DB, Ades PA, Nair KS. Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans. *Am.J.Physiol.Endocrinol.Metab.* 1997;**273**:E790-800.

104. 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.USA* 1996;**93**:15364-9.

105. Craig R. The structure of the contractile filaments. In Engel AG, Frauziori-Armstrong C, eds. pp 134-75. New York: McGraw-Hill, Inc, 1994.

106. Balagopal P, Nair KS, Stirewalt WS. Isolation of myosin heavy chain from small skeletal muscle samples by preparative continuous elution gel electrophoresis: application to measurement of synthesis rate in human and animal tissue. *Anal.Biochem.* 2001;**221**:72-7.

107. Balagopal P, Ljungqvist O, Nair KS. Skeletal muscle myosin heavy-chain synthesis rate in healthy humans. *Am.J.Physiol.Endocrinol.Metab.* 1997;**272**:E45-50.

108. Balagopal P, Ford GC, Ebenstein DB, Nadeau DA, Nair KS. Mass spectrometric methods for determination of [^{13}C] Leucine enrichment in human muscle protein. *Anal.Biochem.* 1996;**239**:77-85.

109. Harman D. Aging: A theory based on free radical and radiation chemistry. *J.Gerontol.* 1956;**11**:300.

110. Hornsby PJ. Current challenges for DHEA research. *Ann.NY.Acad.Sci.* 1995;**774**:xiii-xiv.

111. Buffington CK, Pourmotabbed G, Kitabchi AE. Case report: amelioration of insulin resistance in diabetes with dehydroepiandrosterone. *Am.J.Med.Sci.* 1993;**306**:320-4.

112. Fryburg DA. Insulin-like growth factor I exerts growth hormone- and insulin-like actions on human muscle protein metabolism. *Am.J.Physiol.Endocrinol.Metab.* 1994;**267**:E331-6.

113. 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**:2926-37.

114. Zhang L, Li B, Ma W, Barker JL, Chang YH, Zhao W *et al*. Dehydroepiandrosterone (DHEA) and its sulfated derivative (DHEAS) regulate apoptosis during neurogenesis by triggering the Akt signaling pathway in opposing ways. *Brain Res.Mol.Brain Res.* 2002;**98**:58-66.

115. Yang NC, Jeng KC, Ho WM, Hu ML. ATP depletion is an important factor in DHEA-induced growth inhibition and apoptosis in BV-2 cells. *Life Sci.* 2002;**70**:1979-88.

116. Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J *et al*. Sequence and organization of the human mitochondrial genome. *Nature* 1981;**290**:457-65.

117. Attardi G, Schatz G. Biogenesis of mitochondria. *Annu.Rev.Cell.Biol.* 1988;**4**:289-333.

118. Lardy H, Kneer N, Bellei M, Bobyleva V. Induction of thermogenic enzymes by DHEA and its metabolites. *Ann.NY.Acad.Sci.* 1995;**774**:171-9.

119. Tagliaferro AR, Davis JR, Truchon S, Van Hamont N. Effects of dehydroepiandrosterone acetate on metabolism, body weight and composition of male and female rats. *J.Nutr.* 1986;**116**:1977-83.

120. Wallace DC. Mitochondrial diseases in man and mouse. *Science* 1999;**283**:1482-8.

121. Barazzoni R, Short KR, Nair KS. Effects of aging on mitochondrial DNA copy number and cytochrome c oxidase gene expression in rat skeletal muscle, liver, and heart. *J.Biol.Chem.* 2000;**275**:3343-7.

122. Schon EA, Bonilla E, DiMauro S. Mitochondrial DNA mutations and pathogenesis. *J.Bioenerg.Biomembr.* 1997;**29**:131-49.

123. Polyak K, Li Y, Zhu H, Lengauer C, Willson JK, Markowitz SD *et al*. Somatic mutations of the mitochondrial genome in human colorectal tumours. *Nature Genet.* 1998;**20**:291-3.

124. Chinnery PF, Samuels DC, Elson J, Turnbull DM. Accumulation of mitochondrial DNA mutations in ageing, cancer, and mitochondrial disease: is there a common mechanism? *Lancet* 2002;**360**:1323-5.

125. Sonka J, Hilgertova J, Sevcik J, Kratochvil O. In vitro and in vivo inhibitory effect of dehydroepiandrosterone on respiration. *Endocrinol.Exp.* 1978;**12**:165-9.

126. Mohan PF, Cleary MP. Dehydroepiandrosterone and related steroids inhibit mitochondrial respiration in vitro. *Int.J.Biochem.* 1989;**21**:1103-7.

127. Marrero M, Prough RA, Putnam RS, Bennett M, Milewich L. Inhibition of carbamoyl phosphate synthetase-I by dietary dehydroepiandrosterone. *J.Steroid Biochem.Mol.Biol.* 1991;**38**:599-609.

128. Anderson E, Lee MT, Lee GY. Cystogenesis of the ovarian antral follicle of the rat: ultrastructural changes and hormonal profile following the administration of dehydroepiandrosterone. *Anat.Rec.* 1992;**234**:359-82.

129. Marrero M, Prough RA, Frenkel RA, Milewich L. Dehydroepiandrosterone feeding and protein phosphorylation, phosphatases, and lipogenic enzymes in mouse liver. *Proc.Soc.Exp.Biol.Med.* 1990;**193**:110-7.

130. Miller WL. Early steps in androgen biosynthesis: from cholesterol to DHEA. *Baillieres Clin.Endocrinol.Metab.* 2002;**12**:67-81.

131. Mohan PF, Cleary MP. Short-term effects of dehydroepiandrosterone treatment in rats on mitochondrial respiration. *J.Nutr.* 1991;**121**:240-50.

132. Bobyleva V, Kneer N, Bellei M, Battelli D, Lardy HA. Concerning the mechanism of increased thermogenesis in rats treated with dehydroepiandrosterone. *J.Bioenerg.Biomembr.* 1993;**25**:313-21.

133. Swierczynski J, Mayer D. Dehydroepiandrosterone-induced lipid peroxidation in rat liver mitochondria. *J.Steroid Biochem.Mol.Biol.* 1996;**58**:599-603.

134. Wu G. An important role for pentose cycle in the synthesis of citrulline and proline from glutamine in porcine enterocytes. *Arch.Biochem.Biophys.* 1996;**336**:224-30.

135. Bobyleva V, Bellei M, Kneer N, Lardy H. The effects of the ergosteroid 7-oxo-dehydroepiandrosterone on mitochondrial membrane potential: possible relationship to thermogenesis. *Arch.Biochem.Biophys.* 1997;**341**:122-8.

136. Short KR, Nygren J, Barazzoni R, Levine J, Nair KS. T(3) increases mitochondrial ATP production in oxidative muscle despite increased expression of UCP2 and -3. *Am.J.Physiol.Endocrinol.Metab.* 2001;**280**:E761-9.

137. Ryu JW, Kim MS, Kim CH, Song KH, Park JY, Lee JD *et al*. DHEA administration increases brown fat uncoupling protein 1 levels in obese OLETF rats. *Biochem.Biophys.Res.Commun.* 2003;**303**:726-31.

138. Berdanier CD, Everts HB, Hermoyian C, Mathews CE. Role of vitamin A in mitochondrial gene expression. *Diabetes Res.Clin.Pract.* 2001;**54**:S11-27.

139. Waxman DJ. Role of metabolism in the activation of dehydroepiandrosterone as a peroxisome proliferator. *J.Endocrinol.* 1996;**150**:S129-47.

140. Frenkel RA, Slaughter CA, Orth K, Moomaw CR, Hicks SH, Snyder JM *et al*. Peroxisome proliferation and induction of peroxisomal enzymes in mouse and rat liver by dehydroepiandrosterone feeding. *J.Steroid Biochem.* 1990;**35**:333-42.

141. Mastrocola R, Aragno M, Betteto S, Brignardello E, Catalano MG, Danni O *et al*. Pro-oxidant effect of dehydroepiandrosterone in rats is mediated by PPAR activation. *Life Sci.* 2003;**73**:289-99.

142. Chance DS, Wu SM, McIntosh MK. Inverse relationship between peroxisomal and mitochondrial beta-oxidation in HepG2 cells treated with dehydroepiandrosterone and clofibric acid. *Proc.Soc.Exp.Biol.Med.* 1995;**208**:378-84.

143. Metzger C, Mayer D, Hoffmann H, Bocker T, Hobe G, Benner A *et al*. Sequential appearance and ultrastructure of amphophilic cell foci, adenomas, and carcinomas in the liver of male and female rats treated with dehydroepiandrosterone. *Toxicol.Pathol.* 1995;**23**:591-605.

144. Swierczynski J, Kochan Z, Mayer D. Dietary alpha-tocopherol prevents dehydroepiandrosterone-induced lipid peroxidation in rat liver microsomes and mitochondria. *Toxicol.Lett.* 1997;**91**:129-36.

145. Hoppener JW, de Pagter-Holthuizen P, Geurts van Kessel AH, Jansen M, Kittur SD, Antonarakis SE *et al*. The human gene encoding insulin-like growth factor I is located on chromosome 12. *Hum.Genet.* 1985;**69**:157-60.

146. Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. *Endocr.Rev.* 1995;**16**:3-34.

147. Juul A, Bang P, Hertel NT, Main K, Dalgaard P, Jorgensen K *et al*. Serum insulin-like growth factor-I in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size, and body mass index. *J.Clin.Endocrinol.Metab.* 1994;**78**:744-52.

148. Yu H, Mistry J, Nicar MJ, Khosravi MJ, Diamandis A, Van Doorn J *et al*. Insulin-like growth factors (IGF-I, free IGF-I and IGF-II) and insulin-like

growth factor binding proteins (IGFBP-2, IGFBP-3, IGFBP-6, and ALS) in blood circulation. *J.Clin.Lab.Anal.* 1999;**13**:166-72.

149. Hong Y, Pedersen NL, Brismar K, Hall K, de Faire U. Quantitative genetic analyses of insulin-like growth factor I (IGF-I), IGF-binding protein-1, and insulin levels in middle-aged and elderly twins. *J.Clin.Endocrinol.Metab.* 1996;**81**:1791-7.

150. Harrela M, Koistinen H, Kaprio J, Lehtovirta M, Tuomilehto J, Eriksson J *et al*. Genetic and environmental components of interindividual variation in circulating levels of IGF-I, IGF-II, IGFBP-1, and IGFBP-3. *J.Clin.Invest.* 1996;**98**:2612-5.

151. de Pagter-Holthuizen P, van Schaik FM, Verduijn GM, van Ommen GJ, Bouma BN, Jansen M *et al*. Organization of the human genes for insulin-like growth factors I and II. *FEBS Lett.* 1986;**195**:179-84.

152. Gelato MC, Frost RA. IGFBP-3. Functional and structural implications in aging and wasting syndromes. *Endocrine* 1997;**7**:81-5.

153. Baxter RC, Martin JL, Beniac VA. High molecular weight insulin-like growth factor binding protein complex. Purification and properties of the acid-labile subunit from human serum. *J.Biol.Chem.* 1989;**264**:11843-8.

154. Guler HP, Zapf J, Schmid C, Froesch ER. Insulin-like growth factors I and II in healthy man. Estimations of half-lives and production rates. *Acta Endocrinol.(Copenhagen)* 1989; **121**:753-8.

155. Alitalo T, Kontula K, Koistinen R, Aalto-Setala K, Julkunen M, Janne OA *et al*. The gene encoding human low-molecular weight insulin-like growth-factor binding protein (IGF-BP25): regional localization to 7p12-p13 and description of a DNA polymorphism. *Hum.Genet.* 1989;**83**:335-8.

156. Suikkari AM, Koivisto VA, Rutanen EM, Yki-Jarvinen H, Karonen SL, Seppala M. Insulin regulates the serum levels of low molecular weight insulin-like growth factor-binding protein. *J.Clin.Endocrinol.Metab.* 1988;**66**:266-72.

157. Holly JM, Biddlecombe RA, Dunger DB, Edge JA, Amiel SA, Howell R *et al*. Circadian variation of GH-independent IGF-binding protein in diabetes mellitus and its relationship to insulin. A new role for insulin. *Clin.Endocrinol.(Oxf)* 1988;**29**:667-75.

158. Brismar K, Fernqvist-Forbes E, Wahren J, Hall K. Effect of insulin on the hepatic production of insulin-like growth factor-binding protein-1 (IGFBP-1), IGFBP-3, and IGF-I in insulin-dependent diabetes. *J.Clin.Endocrinol.Metab.* 1994;**79**:872-8.

159. Nam SY, Lee EJ, Kim KR, Cha BS, Song YD, Lim SK *et al*. Effect of obesity on total and free insulin-like growth factor (IGF)-1, and their relationship to IGF-binding protein (BP)-1, IGFBP-2, IGFBP-3, insulin, and growth hormone. *Int.J.Obes.Relat.Metab.Disord.* 1997;**21**:355-9.

160. Hintz RL, Liu F, Rosenfeld RG, Kemp SF. Plasma somatomedin-binding proteins in hypopituitarism: changes during growth hormone therapy. *J.Clin.Endocrinol.Metab.* 1981;**53**:100-4.

161. Juul A, Pedersen SA, Sorensen S, Winkler K, Jorgensen JO, Christiansen JS *et al*. Growth hormone (GH) treatment increases serum insulin-like growth factor binding protein-3, bone isoenzyme alkaline phosphatase and forearm bone mineral content in young adults with GH deficiency of childhood onset. *Eur.J.Endocrinol.* 1994;**131**:41-9.

162. Villareal DT, Holloszy JO, Kohrt WM. Effects of DHEA replacement on bone mineral density and body composition in elderly women and men. *Clin.Endocrinol.(Oxf)* 2000;**53**:561-8.

163. Fottner C, Engelhardt D, Weber MM. Regulation of steroidogenesis by insulin-like growth factors (IGFs) in adult human adrenocortical cells: IGF-I and, more potently, IGF-II preferentially enhance androgen biosynthesis through interaction with the IGF-I receptor and IGF-binding proteins. *J.Endocrinol.* 2001;**158**:409-17.

164. Casson PR, Santoro N, Elkind-Hirsch K, Carson SA, Hornsby PJ, Buster JE *et al*. Postmenopausal dehydroepiandrosterone administration increases free insulin-like growth factor-I and decreases high-density lipoprotein: a six-month trial. *Fertil.Steril.* 1998;**70**:107-10.

165. Morales AJ, Haubrich RH, Hwang JY, Asakura H, Yen SS. The effect of six months treatment with a 100 mg daily dose of dehydroepiandrosterone (DHEA) on circulating sex steroids, body composition and muscle strength in age-advanced men and women. *Clin.Endocrinol.(Oxf)* 1998;**49**:421-32.

166. Genazzani AD, Stomati M, Strucchi C, Puccetti S, Genazzani AR, Luisi S. Oral dehydroepiandrosterone supplementation modulates spontaneous and growth hormone-releasing hormone-induced growth hormone and insulin-like growth factor-1 secretion in early and late postmenopausal women. *Fertil.Steril.* 2001;**76**:241-8.

167. Tissandier O, Peres G, Fiet J, Piette F. Testosterone, dehydroepiandrosterone, insulin-like growth factor 1, and insulin in sedentary and physically trained aged men. *Eur.J.Appl.Physiol.* 2001;**85**:177-84.

168. Chadan SG, Dill RP, Vanderhoek K, Parkhouse WS. Influence of physical activity on plasma insulin-like growth factor - 1 and insulin like growth factor binding proteins in healthy older women. *Mech.Ageing Dev.* 1999;**109**:21-34.

169. Awede B, Thissen JP, Gailly P, Lebacq J. Regulation of IGF 1, IGFBP 4 and IGFBP 5 gene expression by loading in mouse skeletal muscle. *FEBS Lett.* 1999;**461**:263-7.

170. Eliakim A, Moromisato M, Moromisato D, Brasel JA, Roberts CJ, Cooper DM. Increase in muscle IGF-I protein but not IGF-I mRNA after 5 days of

endurance training in young rats. *Am.J.Physiol.Regul.Integr.Comp.Physiol.* 1997;**273**:R1557-61.

171. Feek CM, Ratcliffe JG, Seth J, Gray CE, Toft AD, Irvine WJ. Patterns of plasma cortisol and ACTH concentrations in patients with Addison's disease treated with conventional corticosteroid replacement. *Clin.Endocrinol.(Oxf)* 1981;**14**:451-8.

172. Crosby PD, Rittmaster RS. Predictors of clinical response in hirsute women treated with spironolactone. *Fertil.Steril.* 1991;**55**:1076-81.

173. Tidd MJ, Horth CE, Ramsay LE, Shelton JR, Palmer RF. Endocrine effects of spironolactone in man. *Clin.Endocrinol.(Oxf)* 1978;**9**:389-99.

174. Heuser I, Deuschle M, Weber B, Stalla GK, Holsboer F. Increased activity of the hypothalamus pituitary adrenal system after treatment with the mineralocorticoid receptor antagonist spironolactone. *Psychoneuroendocrinology* 2000;**25**:513-8.

175. Borg GA. Psychophysical bases of perceived exertion. *Med.Sci.Sports Exerc.* 1982;**14**:377-81.

176. Proctor DN, Beck KC. Delay time adjustments to minimize errors in breath-by-breath measurement of VO_2 during exercise. *J.Appl.Physiol.* 1996;**81**:2495-9.

177. Weir JB. A new method for calculating metabolic rate with special reference to protein metabolism. *J.Physiol.* 1949;**109**:1-9.

178. Livesey G, Elia M. Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: evaluation of errors with special reference to the detailed composition of fuels. *Am.J.Clin.Nutr.* 1988;**47**:608-28.

179. Abumrad NN, Rabin D, Diamond MP, Lacy WW. Use of a heated superficial hand vein as an alternative site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. *Metabolism* 1981;**30**:936-40.

180. Bergstrom J. Muscle electrolytes in man: determined by neutron activation analysis on needle biopsy specimen: a study in normal subjects, kidney patients, and patients with chronic diarrhoea. *Scand.J.Clin.Lab.Invest.* 1962;**14**:11-3.

181. Edwards RH. Percutaneous needle-biopsy of skeletal muscle in diagnosis and research. *Lancet* 1971;**2**:593-5.

182. Evans WJ, Phinney SD, Young VR. Suction applied to a muscle biopsy maximizes sample size. *Med.Sci.Sports Exerc.* 1982;**14**:101-2.

183. Jones BN, Gilligan JP. o-Phthaldialdehyde precolumn derivatization and reversed-phase high-performance liquid chromatography of polypeptide hydrolysates and physiological fluids. *J.Chromatogr.A* 1983;**266**:471-82.

184. Beer NA, Jakubowicz DJ, Beer RM, Arocha IR, Nestler JE. Effects of nitrendipine on glucose tolerance and serum insulin and dehydroepiandrosterone sulfate levels in insulin-resistant obese and hypertensive men. *J.Clin.Endocrinol.Metab.* 1993;**76**:178-83.

185. Rosenfeld RG, Lamson G, Pham H, Oh Y, Conover C, De Leon DD *et al*. Insulin like growth factor-binding proteins. *Rec.Progr.Horm.Res.* 1990;**46**:99-163.

186. Lee, P D, Powell, D, Baker, B, Liu, F, Mathew, G, Levitsky, I, Gutierrez, O D, and Hintz, R L. Characterization of a direct, non-extraction immunoradiometric assay for free IGF-1. Presented at the 76th annual meeting of the Endocrine Society.Anaheim, CA . 1994.

187. Underwood LE, Murphy MG. Radioimmunoassay of the somatomedins. In Patrono C, ed. *Radioimmunoassay in Basic and Clinical Pharmacology (Handbook of Experimental Pharmacology. Vol 82)*, pp 561-74. Heidelberg: Springer-Verlong, 1987.

188. Conover CA, Lee PD, Kanaley JA, Clarkson JT, Jensen MD. Insulin regulation of insulin-like growth factor binding protein-1 in obese and nonobese humans. *J.Clin.Endocrinol.Metab.* 1992;**74**:1355-60.

189. Hammond GL, Langley MS, Robinson PA. A liquid-phase immunoradiometric assay (IRMA) for human sex hormone binding globulin (SHBG). *J.Steroid Biochem.* 1985;**23**:451-60.

190. Rooyackers OE, Balagopal P, Nair KS. Measurement of synthesis rates of specific muscle proteins using needle biopsy samples. *Muscle Nerve* 1998;**20**:S93-6.

191. Krieger DA, Tate CA, McMillin-Wood J, Booth FW. Populations of rat skeletal muscle mitochondria after exercise and immobilization. *J.Appl.Physiol.* 1980;**48**:23-8.

192. Fischer JC, Ruitenbeek W, Stadhouders AM, Trijbels JM, Sengers RC, Janssen AJ *et al*. Investigation of mitochondrial metabolism in small human skeletal muscle biopsy specimens. Improvement of preparation procedure. *Clin.Chim.Acta* 1985;**145**:89-99.

193. Rooyackers OE, Senden JM, Soeters PB, Saris WH, Wagenmakers AJ. Prolonged activation of the branched-chain alpha-keto acid dehydrogenase complex in muscle of zymosan treated rats. *Eur.J.Clin.Invest.* 1995;**25**:548-52.

194. Ljungqvist OH, Persson M, Ford GC, Nair KS. Functional heterogeneity of leucine pools in human skeletal muscle. *Am.J.Physiol.Endocrinol.Metab.* 1997;**273**:E564-70.

195. Nair KS. Assessment of protein metabolism in diabetes. In Nair KS, ed. *Methods, assessment, and metabolic regulation*, pp 137-70. Totowa, NJ: Humana Press, Inc., 1997.

196. Christiansen, J J, Gravholt, C H, Fisker, S, Andersen, M, Svendstrup, B, and Christiansen, J S. Short term restoration of androgen levels with dehydroepiandrosterone (DHEA) in female adrenal insufficiency does not affect insulin sensitivity. Abstract presented at The Endocrine Society Meeting, San Francisco . 2002.
Ref Type: Abstract

197. Diamond P, Cusan L, Gomez JL, Belanger A, Labrie F. Metabolic effects of 12-month percutaneous dehydroepiandrosterone replacement therapy in postmenopausal women. *J.Endocrinol.* 1996;**150**:S43-50.

198. Jakubowicz D, Beer N, Rengifo R. Effect of dehydroepiandrosterone on cyclic-guanosine monophosphate in men of advancing age. *Ann.NY.Acad.Sci.* 1995;**774**:312-5.

199. Lasco A, Frisina N, Morabito N, Gaudio A, Morini E, Trifiletti A *et al*. Metabolic effects of dehydroepiandrosterone replacement therapy in postmenopausal women. *Eur.J.Endocrinol.* 2001;**145**:457-61.

200. Vogiatzi MG, Boeck MA, Vlachopapadopoulou E, el-Rashid R, New MI. Dehydroepiandrosterone in morbidly obese adolescents: effects on weight, body composition, lipids, and insulin resistance. *Metabolism* 1996;**45**:1011-5.

201. Usiskin KS, Butterworth S, Clore JN, Arad Y, Ginsberg HN, Blackard WG *et al*. Lack of effect of dehydroepiandrosterone in obese men. *Int.J.Obes.* 1990; **14**:457-63.

202. Nestler JE, Barlascini CO, Clore JN, Blackard WG. Dehydroepiandrosterone reduces serum low density lipoprotein levels and body fat but does not alter insulin sensitivity in normal men. *J.Clin.Endocrinol.Metab.* 1988;**66**:57-61.

203. Kawano H, Yasue H, Kitagawa A, Hirai N, Yoshida T, Soejima H *et al*. Dehydroepiandrosterone supplementation improves endothelial function and insulin sensitivity in men. *J Clin Endocrinol Metab* 2003;**88**:3190-5.

204. Gurnell, E M, Hunt, P J, Curran, S E, Conway, C L, Huppert, F A, Herbert, J, and Chatterjee, K K. A long term trial of DHEA replacement in Addison's disease. Abstract presented at The Endocrine Society Meeting, San Francisco . 2002.

205. Basu R, Breda E, Oberg AL, Powell CC, Dalla Man CD, Basu A *et al*. Mechanisms of the age-associated deterioration in glucose tolerance: contribution of alterations in insulin secretion, action and clearance. *Diabetes* 2003;**52**:1738-48.

206. Schriock ED, Buffington CK, Hubert GD, Kurtz BR, Kitabchi AE, Buster JE *et al*. Divergent correlations of circulating dehydroepiandrosterone sulfate and

testosterone with insulin levels and insulin receptor binding. *J.Clin.Endocrinol.Metab.* 1988;**66**:1329-31.

207. Paolisso G, Ammendola S, Rotondi M, Gambardella A, Rizzo MR, Mazziotti G *et al*. Insulin resistance and advancing age: what role for dehydroepiandrosterone sulfate? *Metabolism* 1997;**46**:1281-6.

208. Barrett-Connor E. Lower endogenous androgen levels and dyslipidemia in men with non-insulin-dependent diabetes mellitus. *Ann.Intern.Med.* 1992;**117**:807-11.

209. Herranz L, Megia A, Grande C, Gonzalez-Gancedo P, Pallardo F. Dehydroepiandrosterone sulphate, body fat distribution and insulin in obese men. *Int.J.Obes.Relat.Metab.Disord.* 1995;**19**:57-60.

210. Mortola JF, Yen SS. The effects of oral dehydroepiandrosterone on endocrine-metabolic parameters in postmenopausal women. *J.Clin.Endocrinol.Metab.* 1990;**71**:696-704.

211. Barquist E, Kirton O. Adrenal insufficiency in the surgical intensive care unit patient. *J.Trauma* 1997;**42**:27-31.

212. Van den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M *et al*. Intensive insulin therapy in the surgical intensive care unit. *N.Eng.J.Med.* 2001;**345**:1359-67.

213. Bavenholm PN, Pigon J, Efendic S. Insulin sensitivity of suppression of endogenous glucose production is the single most important determinant of glucose tolerance. *Diabetes* 2001;**50**:1449-54.

214. Fendri S, Roussel B, Lormeau B, Tribout B, Lalau JD. Insulin sensitivity, insulin action, and fibrinolysis activity in nondiabetic and diabetic obese subjects. *Metabolism* 1998;**47**:1372-5.

215. Lewis GF, Carpentier A, Adeli K, Giacca A. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocr.Rev.* 2002;**23**:201-29.

216. Nakashima N, Haji M, Sakai Y, Ono Y, Umeda F, Nawata H. Effect of dehydroepiandrosterone on glucose uptake in cultured human fibroblasts. *Metabolism* 1995;**44**:543-8.

217. De Pergola G. The adipose tissue metabolism: role of testosterone and dehydroepiandrosterone. *Int.J.Obes.Relat.Metab.Disord.* 2000;**24**:S59-63.

218. Buffington CK, Givens JR, Kitabchi AE. Opposing actions of dehydroepiandrosterone and testosterone on insulin sensitivity. In vivo and in vitro studies of hyperandrogenic females. *Diabetes* 1991;**40**:693-700.

219. Ishizuka T, Kajita K, Miura A, Ishizawa M, Kanoh Y, Itaya S *et al*. DHEA improves glucose uptake via activations of protein kinase C and

phosphatidylinositol 3-kinase. *Am.J.Physiol.Endocrinol.Metab.* 1999;**276**:E196-E204.

220. Kajita K, Ishizuka T, Miura A, Ishizawa M, Kanoh Y, Yasuda K. The role of atypical and conventional PKC in dehydroepiandrosterone-induced glucose uptake and dexamethasone-induced insulin resistance. *Biochem.Biophys.Res.Commun.* 2000;**277**:361-7.

221. Paolisso G, Tagliamonte MR, Rizzo MR, Carella C, Gambardella A, Barbieri M *et al*. Low plasma insulin-like growth factor-1 concentrations predict worsening of insulin-mediated glucose uptake in older people. *J.Am.Geriatr.Soc.* 1999;**47**:1312-8.

222. Aoki K, Nakajima A, Mukasa K, Osawa E, Mori Y, Sekihara H. Prevention of diabetes, hepatic injury, and colon cancer with dehydroepiandrosterone. *J.Steroid Biochem.Mol.Biol.* 2003;**85**:469-72.

223. Christiansen JJ, Gravholt CH, Fisker S, Svendstrup B, Bennett P, Veldhuis JD *et al*. Dehydroepiandrosterone supplementation in women with adrenal failure: impact on twenty-four hour GH secretion and IGF-related parameters. *Clin.Endocrinol.(Oxf)* 2004;**60**:461-9.

224. Johannsson G, Burman P, Wiren L, Engstrom BE, Nilsson AG, Ottosson M *et al*. Low dose dehydroepiandrosterone affects behavior in hypopituitary androgen-deficient women: a placebo-controlled trial. *J.Clin.Endocrinol.Metab.* 2002;**87**:2046-52.

225. Benbassat CA, Maki KC, Unterman TG. Circulating levels of insulin-like growth factor (IGF) binding protein-1 and -3 in aging men: relationships to insulin, glucose, IGF, and dehydroepiandrosterone sulfate levels and anthropometric measures. *J.Clin.Endocrinol.Metab.* 1997;**82**:1484-91.

226. Abbasi A, Duthie EH, Sheldahl L, Wilson C, Sasse E, Rudman I *et al*. Association of dehydroepiandrosterone sulfate, body composition, and physical fitness in independent community-dwelling older men and women. *J.Am.Geriatr.Soc.* 1998;**46**:263-73.

227. Haden ST, Glowacki J, Hurwitz S, Rosen C, LeBoff MS. Effects of age on serum dehydroepiandrosterone sulfate, IGF-I, and IL-6 levels in women. *Calcif.Tissue Int.* 2000;**66**:414-8.

228. Morley JE, Kaiser F, Raum WJ, Perry HM, Flood JF, Jensen J *et al*. Potentially predictive and manipulable blood serum correlates of aging in the healthy human male: progressive decreases in bioavailable testosterone, dehydroepiandrosterone sulfate, and the ratio of insulin-like growth factor 1 to growth hormone. *Proc.Natl.Acad.Sci.USA* 1997;**94**:7537-42.

229. Genazzani AD, Stomati M, Bernardi F, Pieri M, Rovati L, Genazzani AR. Long-term low-dose dehydroepiandrosterone oral supplementation in early and late postmenopausal women modulates endocrine parameters and synthesis of neuroactive steroids. *Fertil.Steril.* 2003;**80**:1495-501.

230. Janssen JA, Stolk RP, Pols HA, Grobbee DE, Lamberts SW. Serum total IGF-I, free IGF-I, and IGFB-1 levels in an elderly population: relation to cardiovascular risk factors and disease. *Arterioscler.Thromb.Vasc.Biol.* 1998;**18**:277-82.

231. Slowinska-Srzednicka J, Zgliczynski S, CiSwicka-Sznajderman M, Srzednicki M, Soszynski P, Biernacka M *et al*. Decreased plasma dehydroepiandrosterone sulfate and dihydrotestosterone concentrations in young men after myocardial infarction. *Atherosclerosis* 1989;**79**:197-203.

232. Nestler JE, Usiskin KS, Barlascini CO, Welty DF, Clore JN, Blackard WG. Suppression of serum dehydroepiandrosterone sulfate levels by insulin: an evaluation of possible mechanisms. *J.Clin.Endocrinol.Metab.* 1989;**69**:1040-6.

233. Barnhart KT, Freeman E, Grisso JA, Rader DJ, Sammel M, Kapoor S *et al*. The effect of dehydroepiandrosterone supplementation to symptomatic perimenopausal women on serum endocrine profiles, lipid parameters, and health-related quality of life. *J.Clin.Endocrinol.Metab.* 1999;**84**:3896-902.

234. Barrett-Connor E, Khaw KT. Endogenous sex hormones and cardiovascular disease in men. A prospective population-based study. *Circulation* 1998;**78**:539-45.

235. Barrett-Connor E, Goodman-Gruen D. Dehydroepiandrosterone sulfate does not predict cardiovascular death in postmenopausal women. The Rancho Bernardo Study. *Circulation* 1995;**91**:1757-60.

236. Feldman HA, Johannes CB, Araujo AB, Mohr BA, Longcope C, McKinlay JB. Low dehydroepiandrosterone and ischemic heart disease in middle-aged men: prospective results from the Massachusetts Male Aging Study. *Am.J.Epidemiol.* 2001;**153**:79-89.

237. Barrett-Connor E, Ferrara A. Dehydroepiandrosterone, dehydroepiandrosterone sulfate, obesity, waist-hip ratio, and noninsulin-dependent diabetes in postmenopausal women: the Rancho Bernardo Study. *J.Clin.Endocrinol.Metab.* 1996;**81**:59-64.

238. Johannes CB, Stellato RK, Feldman HA, Longcope C, McKinlay JB. Relation of dehydroepiandrosterone and dehydroepiandrosterone sulfate with cardiovascular disease risk factors in women: longitudinal results from the Massachusetts Women's Health Study. *J.Clin.Epidemiol.* 1999;**52**:95-103.

239. Deeb SS, Fajas L, Nemoto M, Pihlajamaki J, Mykkanen L, Kuusisto J *et al*. A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nature Genet.* 1998;**20**:284-7.

240. Bonnet S, Dumas-de-La-Roque E, Begueret H, Marthan R, Fayon M, Dos Santos P *et al*. Dehydroepiandrosterone (DHEA) prevents and reverses chronic hypoxic pulmonary hypertension. *Proc.Natl.Acad.Sci.USA* 2003;**100**:9488-93.

241. Spallarossa P, Brunelli C, Minuto F, Caruso D, Battistini M, Caponnetto S *et al*. Insulin-like growth factor-I and angiographically documented coronary artery disease. *Am.J.Cardiol.* 1996;**77**:200-2.

242. Ferns GA, Motani AS, Anggard EE. The insulin-like growth factors: their putative role in atherogenesis. *Artery* 1991;**18**:197-225.

243. Froesch ER, Zenobi PD, Hussain M. Metabolic and therapeutic effects of insulin-like growth factor I. *Horm.Res.* 1994;**42**:66-71.

244. Tsukahara H, Gordienko DV, Tonshoff B, Gelato MC, Goligorsky MS. Direct demonstration of insulin-like growth factor-I-induced nitric oxide production by endothelial cells. *Kidney Int.* 1994;**45**:598-604.

245. LeRoith D, Werner H, Beitner-Johnson D, Roberts CT. Molecular and cellular aspects of the insulin-like growth factor I receptor. *Endocr.Rev.* 1995;**16**:143-63.

246. Walsh MF, Barazi M, Pete G, Muniyappa R, Dunbar JC, Sowers JR. Insulin-like growth factor I diminishes in vivo and in vitro vascular contractility: role of vascular nitric oxide. *Endocrinology* 1996;**137**:1798-803.

247. Radomski MW, Salas E. Nitric oxide--biological mediator, modulator and factor of injury: its role in the pathogenesis of atherosclerosis. *Atherosclerosis* 1995;**118**:S69-80.

248. Trivedi DP, Khaw KT. Dehydroepiandrosterone sulfate and mortality in elderly men and women. *J Clin Endocrinol Metab* 2001;**86**:4171-7.

249. Flynn MA, Weaver-Osterholtz D, Sharpe-Timms KL, Allen S, Krause G. Dehydroepiandrosterone replacement in aging humans. *J.Clin.Endocrinol.Metab.* 1999;**84**:1527-33.

250. Kostka T, Arsac LM, Patricot MC, Berthouze SE, Lacour JR, Bonnefoy M. Leg extensor power and dehydroepiandrosterone sulfate, insulin-like growth factor-I and testosterone in healthy active elderly people. *Eur.J.Appl.Physiol.* 2000;**82**:83-90.

251. Kurzman ID, MacEwen EG, Haffa AL. Reduction in body weight and cholesterol in spontaneously obese dogs by dehydroepiandrosterone. *Int.J.Obes.* 1990;**14**:95-104.

252. Maccario M, Mazza E, Ramunni J, Oleandri SE, Savio P, Grottoli S *et al*. Relationships between dehydroepiandrosterone-sulphate and anthropometric, metabolic and hormonal variables in a large cohort of obese women. *Clin.Endocrinol.(Oxf)* 1999;**50**:595-600.

253. Welle S, Jozefowicz R, Statt M. Failure of dehydroepiandrosterone to influence energy and protein metabolism in humans. *J.Clin.Endocrinol.Metab.* 1990;**71**:1259-64.

254. Percheron G, Hogrel JY, Denot-Ledunois S, Fayet G, Forette F, Baulieu EE. Effect of 1-year oral administration of dehydroepiandrosterone to 60- to 80-year-old individuals on muscle function and cross-sectional area: a double-blind placebo-controlled trial. *Arch.Intern.Med.* 2003;**163**:720-7.

255. Payette H, Roubenoff R, Jacques P, Dinarello C, Wilson P, Abad L *et al*. Insulin-like growth factor-1 and interleukin 6 predict sarcopenia in very old community-living men and women: the Framingham Heart Study. *J.Am.Geriatr.Soc.* 2003;**51**:1237-43.

256. Gordon CM, Grace E, Emans SJ, Feldman HA, Goodman E, Becker KA *et al*. Effects of oral dehydroepiandrosterone on bone density in young women with anorexia nervosa: a randomized trial. *J Clin Endocrinol Metab* 2002;**87** :4935-41.

257. Turner RT, Lifrak ET, Beckner M, Wakley GK, Hannon KS, Parker LN. Dehydroepiandrosterone reduces cancellous bone osteopenia in ovariectomized rats. *Am.J.Physiol.Endocrinol.Metab.* 1990;**258**:E673-7.

258. Boonen S, Vanderschueren D, Cheng XG, Verbeke G, Dequeker J, Geusens P *et al*. Age-related (type II) femoral neck osteoporosis in men: biochemical evidence for both hypovitaminosis D- and androgen deficiency-induced bone resorption. *J.Bone Miner.Res.* 1997;**12**:2119-26.

259. Conte FA, Grumbach MM, Ito Y, Fisher CR, Simpson ER. A syndrome of female pseudohermaphrodism, hypergonadotropic hypogonadism, and multicystic ovaries associated with missense mutations in the gene encoding aromatase (P450arom). *J.Clin.Endocrinol.Metab.* 1994;**78**:1287-92.

260. Smith EP, Boyd J, Frank GR, Takahashi H, Specker B, Williams TC *et al*. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *N.Eng.J.Med.* 1994;**331**:1056-61.

261. Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin K. Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. *J.Clin.Endocrinol.Metab.* 1995;**80**:3689-98.

262. Nawata H, Tanaka S, Tanaka S, Takayanagi R, Sakai Y, Yanase T *et al*. Aromatase in bone cell: association with osteoporosis in postmenopausal women. *J.Steroid Biochem.Mol.Biol.* 1995;**53**:165-74.

263. Takayanagi R, Goto K, Suzuki S, Tanaka S, Shimoda S, Nawata H. Dehydroepiandrosterone (DHEA) as a possible source for estrogen formation in bone cells: correlation between bone mineral density and serum DHEA-sulfate concentration in postmenopausal women, and the presence of aromatase to be enhanced by 1,25-dihydroxyvitamin D3 in human osteoblasts. *Mech.Ageing Dev.* 2002;**123**:1107-14.

264. Clarke BL, Ebeling PR, Jones JD, Wahner HW, O'Fallon WM, Riggs BL *et al*. Predictors of bone mineral density in aging healthy men varies by skeletal site. *Calcif.Tissue Int.* 2002;**70**:137-45.

265. Nordin BE, Robertson A, Seamark RF, Bridges A, Philcox JC, Need AG *et al*. The relation between calcium absorption, serum dehydroepiandrosterone, and vertebral mineral density in postmenopausal women. *J.Clin.Endocrinol.Metab.* 1985;**60**:651-7.

266. Barrett-Connor E, Kritz-Silverstein D, Edelstein SL. A prospective study of dehydroepiandrosterone sulfate (DHEAS) and bone mineral density in older men and women. *Am.J.Epidemiol.* 1993;**137**:201-6.

267. Arlt W, Callies F, Koehler I, van Vlijmen JC, Fassnacht M, Strasburger CJ *et al*. Dehydroepiandrosterone supplementation in healthy men with an age-related decline of dehydroepiandrosterone secretion. *J.Clin.Endocrinol.Metab.* 2001;**86**:4686-92.

268. Kahn AJ, Halloran B. Dehydroepiandrosterone supplementation and bone turnover in middle-aged to elderly men. *J.Clin.Endocrinol.Metab.* 2002;**87**:1544-9.

269. Labrie F, Diamond P, Cusan L, Belanger A, Candas B. Effect of 12-month dehydroepiandrosterone replacement therapy on bone, vagina, and endometrium in postmenopausal women. *J.Clin.Endocrinol.Metab.* 1997;**82**:3498-505.

270. Baulieu EE, Thomas G, Legrain S, Lahlou N, Roger M, Debuire B *et al*. Dehydroepiandrosterone (DHEA), DHEA sulfate, and aging: contribution of the DHEAge study to a sociobiomedical issue. *Proc.Natl.Acad.Sci.USA* 2000;**97**:4279-84.

271. Wallace MB, Lim J, Cutler A, Bucci L. Effects of dehydroepiandrosterone vs androstenedione supplementation in men. *Med.Sci.Sports Exerc.* 1999;**31**:1788-92.

272. Long W, Barrett EJ, Wei L, Liu Z. Adrenalectomy enhances the insulin sensitivity of muscle protein synthesis. *Am.J.Physiol.Endocrinol.Metab.* 2003;**284**:E102-E109.

273. 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.USA* 2003;**100**:7996-8001.

274. Short KR, Vittone JL, Bigelow ML, Proctor DN, Rizza RA, Coenen-Schimke JM *et al*. Impact of aerobic exercise training on age-related changes in insulin sensitivity and muscle oxidative capacity. *Diabetes* 2003;**52**:1888-96.

275. Nagesser SK, van Seters AP, Kievit J, Hermans J, Krans HM, van de Velde CJ. Long-term results of total adrenalectomy for Cushing's disease. *World J.Surg.* 2000;**24**:108-13.

276. Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women.

Principal results from the women's health initiative randomized controlled trial. *JAMA* 2002;**288**:321-33.

277. Couzinet B, Meduri G, Lecce MG, Young J, Brailly S, Loosfelt H *et al*. The postmenopausal ovary is not a major androgen-producing gland. *J.Clin.Endocrinol.Metab.* 2001;**86**:5060-6.

278. Ntoumanis N, Biddle SJ. A review of motivational climate in physical activity. *J.Sports Sci.* 1999;**17**:643-65.

279. Nelson ME, Fiatarone MA, Layne JE, Trice I, Economos CD, Fielding RA *et al*. Analysis of body-composition techniques and models for detecting change in soft tissue with strength training. *Am.J.Clin.Nutr.* 1996;**63**:678-86.

280. Heymsfield SB, Smith R, Aulet M, Bensen B, Lichtman S, Wang J *et al*. Appendicular skeletal muscle mass: measurement by dual-photon absorptiometry. *Am.J.Clin.Nutr.* 1990;**52**:214-8.

281. Munarriz R, Talakoub L, Flaherty E, Gioia M, Hoag L, Kim NN *et al*. Androgen replacement therapy with dehydroepiandrosterone for androgen insufficiency and female sexual dysfunction: androgen and questionnaire results. *J.Sex Marital Ther.* 2002;**28**:165-73.

282. Schwartz AG, Pashko LL. Cancer prevention with dehydroepiandrosterone and non-androgenic structural analogs. *J.Cell.Biochem.Suppl.* 1995;**22**:210-7.

283. Prough RA, Lei XD, Xiao GH, Wu HQ, Geoghegan TE, Webb SJ. Regulation of cytochromes P450 by DHEA and its anticarcinogenic action. *Ann.NY.Acad.Sci.* 1995;**774**:187-99.

284. Parasrampuria J, Schwartz K, Petesch R. Quality control of dehydroepiandrosterone dietary supplement products. *JAMA* 1998;**280**:1565.

285. Sweeney, J E, Osbourne, T. To amend the Controlled Substances Act with respect to the placing of certain substances on the schedules of controlled substances, and for other purposes. HR 207 IH, 107th Congress, House of Representatives, 2nd Session. 10-7-2002.
Ref Type: Unenacted Bill/Resolution

286. Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N.Eng.J.Med.* 2004;**350**:664-71.

287. Bednarek-Tupikowska G, Gosk I, Bohdanowicz-Pawlak A, Kosowska B, Bidzinska B, Milewicz A. Influence of dehydroepiandrosterone on platelet aggregation, superoxide dismutase activity and serum lipid peroxide concentration in rabbits with induced hypercholesterolemia. *Med.Sci.Monit.* 2000;**6**:40-5.

288. Schwarz AJ, Brasel JA, Hintz RL, Mohan S, Cooper DM. Acute effect of brief low- and high-intensity exercise on circulating insulin-like growth factor

(IGF) I, II, and IGF-binding protein-3 and its proteolysis in young healthy men. *J.Clin.Endocrinol.Metab.* 1996;**81**:3492-7.

289. Van Vollenhoven RF. Dehydroepiandrosterone in systemic lupus erythematosus. *Rheum.Dis.Clin.North Am.* 2000;**26**:349-62.

290. Van Vollenhoven RF, Morabito LM, Engleman EG, McGuire JL. Treatment of systemic lupus erythematosus with dehydroepiandrosterone: 50 patients treated up to 12 months. *J.Rheumatol.* 1998;**25**:285-9.