Steroids 106 (2016) 19–25

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Effect of age, gender and exercise on salivary dehydroepiandrosterone circadian rhythm profile in human volunteers

Walid Al-Turk [b], Emad A.S. Al-Dujaili [a,*]

[a] BHF Centre for Cardiovascular Science, University of Edinburgh, Queen's Medical Research Institute, Edinburgh EH16 4JT, Scotland, UK
[b] Faculty of Pharmacy, Middle East University, Amman, Jordan

ARTICLE INFO

Article history:
Received 14 November 2015
Accepted 7 December 2015
Available online 11 December 2015

Keywords:
DHEA
Exercise
Gender
Aging
Circadian rhythm

ABSTRACT

There has been a lot of effort by scientists to elucidate the multi functions of the naturally occurring hormone, dehydroepiandrosterone (DHEA). However, to plan research experiments optimally, it is important first to characterize the diurnal rhythm in healthy individuals. The aim of this research was to investigate the daily circadian rhythms of DHEA among the 2 genders, and the effect of age and exercise on salivary DHEA circadian rhythms. Volunteers (20–39 and 40–60 years) were recruited for 2 studies investigating the salivary DHEA circadian rhythm. The first study looked at the effect of gender and age on DHEA levels on 2 non-consecutive days, and the second study explored the effect of exercise on DHEA circadian rhythm in males. DHEA levels were estimated by a sensitive and specific ELISA method. The results showed a clear daily circadian rhythm in salivary DHEA in all participants groups, however the profile was flatter in the older female group. There was a significant difference between age and gender groups particularly at 8.00 h. In young males DHEA reduced from 541.1 ± 101.3 (mean ± sd) at 8.00 h to 198.9 ± 90.7 pg/mL at 18.00 h; $p < 0.0001$, and young females from 401.6 ± 149.5 to 215.4 ± 95.3 pg/mL; $p < 0.001$. In older males DHEA reduced from 267.5 ± 32.4 to 132.5 ± 46.7 pg/mL; $p < 0.001$, and older females from 147.7 ± 78.1 to 89.5 ± 29.1 pg/mL; $p = 0.05$. DHEA levels on 2 non-consecutive days showed some variations but this was not significant. Aerobic exercise has significantly increased DHEA levels at 2 time points of the day ($p = 0.05$) in male subjects. In conclusion, our study showed a clear daily circadian rhythm in salivary DHEA in all participants was observed, but the profile was flatter in the older groups.

1. Introduction

Dehydroepiandrosterone (DHEA) is one of the most abundant steroid hormones in the human body, and one of the major steroid products in the hypothalamic–pituitary–adrenal axis (HPA), there is a growing need to examine the activity and function of this steroid hormone [1]. DHEA and its sulphated form, dehydroepiandrosterone-sulfate (DHEAS) are the most abundant anabolic steroids present in the circulation produced by the side chain cleavage of 17-hydroxypregnenolone in a reversible unequal equilibrium reaction [2], and converted to dehydroepiandrosterone-sulfate by the enzyme sulfotransferase, and can be reversed back to DHEA. The latter will then be converted to androstenediol and androstenedione. It is excreted in the urine as sulfate and glucuronide conjugates and unconjugated (Free) forms. DHEA is a weak androgen strongly bound to Sex Steroid Binding Globulin and weakly bound to Corticosteroid Binding Globulin and Albumin. DHEA travels in the blood in its sulfated form (DHEAS). Despite the concentration of DHEAS exceeding DHEA by approximately 300–500 times [3], levels of DHEAS in saliva are compromised by the method of entry (ultrafiltration) due to being conjugated and hydrophilic. Therefore, investigating DHEA levels, which enters saliva more abundantly by intracellular diffusion, would be more likely to reflect the concentration of non-protein bound DHEA in plasma [4].

DHEA is one of the first androgens to increase significantly at the onset of adrenarche. Levels increase throughout puberty until adulthood. DHEA is an important precursor to both genders sex steroids (testosterone and estrogens) produced in the adrenal glands and to a minor extent in the gonads and brain. DHEA has now been discovered to possess many important actions in humans and animals. DHEA has been linked to improvement in cognitive function, immune system strengthening and providing an antagonistic action towards the stress hormone, cortisol [5–7].

* Corresponding author at: BHF Centre for Cardiovascular Science University of Edinburgh, Queen's Medical Research Institute, 47 Little France, Edinburgh EH16 4JT, Scotland, UK.
E-mail address: ealdujai@staffmail.ed.ac.uk (E.A.S. Al-Dujaili).

http://dx.doi.org/10.1016/j.steroids.2015.12.001
0039-128X/

In normal physiologic aging the levels of DHEA has been seen to decrease by around 2% per year past the ages of 25–30 to a level of around 20% of the original level by age 75. This drop in DHEA was postulated to cause potential health problems in the elderly and the sufferers of adrenal deficiency. Degradation of DHEA is a natural process of aging associated with the breakdown of proteins related to its production [8]. In recent years, levels of DHEA and its sulfated ester DHEAS have been used as biomarkers in aging and as an additional biomarker of the HPA axis [9–14]. For example, DHEA can be used to quickly identify if the patient is at risk from inflammatory arthritis [15]. The levels of plasma DHEA and DHEAS have been identified as large reservoirs that can be readily converted into more potent androgens throughout the peripheral tissues. The drop in the level of these reservoirs could lead to health issues such as obesity and insulin resistance through the reduced action of glucocorticoid inhibition [16]. This could also be aggravated by the increased levels of cortisol in older ages [17].

Circadian rhythms of adrenal hormones are in response to light/-dark patterns, and respond also to an individual's body clock. Things that may interfere with circadian rhythms are seasons and hours of sunlight day. Seasonal affective disorder is a prime example of how light patterns can significantly disturb circadian rhythms of hormones, which can often end in a disease state. Significant differences in circadian rhythms such as a low level of DHEA with a steady slow decreasing line could be an indication of frailty [18,28]. Higher levels of DHEA have been found to provide benefits against mental conditions such as depression through their antagonistic action against the deleterious effects of cortisol [19]. In addition, DHEA (also known as the "youth hormone") supplementation has been found beneficial for the elderly health and well being. With the decrease in DHEA the level of essential testosterone in the body also decreases to a point where it could be termed an adrenal deficiency which can be harmful to overall health [20].

The role of DHEA in exercise performance has not been fully investigated. However, Aizawa and colleagues [21] found that the use of exercise programmers on rats significantly increased the levels of 5-α-reductase, which is an enzyme indicative of increased function in hormone steroid pathways such as estrogen and testosterone. This could be indicative of increasing the function within muscles and the adrenal cortex, which would sustain a much healthier biochemical profile. Some other studies have reported that aerobic exercise induced weight loss has increased DHEA [22–24]. A study of post-menopausal women found that resistance exercise significantly increased DHEA post exercise, and the levels increased as much as women on HRT [25]. Another study found that aging men that frequently (at least three times a week) participated in moderate-high intensity exercise had higher basal rates of DHEA than other groups, and it increased the participants sleep quality and sexual function [26].

The aim of the study is to investigate the daily circadian rhythms of DHEA among the 2 genders, and the effect of age on salivary DHEA levels and circadian rhythms. In addition, another pilot study will explore the effect of exercise on salivary DHEA levels and daily circadian rhythm. The rationale for the study was that DHEA production like other adrenal steroid hormones secreted in a daily circadian rhythm [27] and can be altered by many factors, but age and gender are the two main factors that affect DHEA production. Studies such as those done by Heaney et al. [28], Erosheva et al. [29], Carlstrom et al. [30] and Ahn et al. [31] used both age and gender as their primary variables. Each study used participants of both sexes with varying ages ranging from 20 to 90 years. Although studies such as the one done by Sulcova et al. [32] have used participants as young as 5 years old, it would not be feasible for us to recruit young subjects.

2. Experimental

2.1. Participants

Following the approval of the project by the University Ethical committee, healthy participants were recruited via the student Digest and Edinburgh University email system. The inclusion criteria of the study were participants between the ages of 20–60 in both the male and female categories. In the first study, DHEA samples would need to be taken over two full non-consecutive days to study the biological variations of DHEA levels in our volunteers. In the second study, male volunteers were recruited to investigate the effect of exercise on DHEA salivary levels. Ingestion of alcohol and/or taking part in strenuous physical activity was prohibited 24 h before and during the collection period. Ingestion of alcohol and participation in high levels of exercise alters the basal DHEA excretion rate which would cause the hormone levels to fluctuate. Non-changeable exclusion criteria were used in the study to prevent any altered and compromising results. Due to the changes in hormone buildup and excretion, pregnant women or those taking Hormone replacement therapies were excluded from the acceptable participants. With the study looking at basal circadian rhythms of DHEA excretion in saliva any hormone changes would alter the final results.

Upon completion of recruitment 32 volunteers were included in the study 1, and 16 in study 2 (all subjects collected samples from 8.00 h up to 22.00 h before exercise and on the exercise day which involved 40 min aerobic exercise, 5 samples were collected). Of the 32 subjects recruited in study 1, all have completed the study and grouped into 2 categories based on their sex into a group of 16 males and 16 females. These groups were further subdivided into 4 further groups based on their age. From the age category of 20–39 years there were 8 males and 8 females. From the 40–60 years age range there were also 8 males and 8 females.

2.2. Sample collection

For study 1, 12 sample collection containers were provided to each participant. Each pot was labeled with the corresponding participant code, time and sample day to ensure a correct collection method. The study required 12 samples, 6 from each of the 2 non-consecutive days during one week, this would allow for a comparison of the individual variation in DHEA levels. The following procedure was used for collection of saliva samples and owing to duplicated nature of the samples, sample times were taken in 2 h intervals to provide an accurate view of the daily circadian rhythm. Sample times were as follows = 08:00, 10:00, 12.00, 14:00, 16:00, and 18:00 h. For study 2, the sample collection was extended up to 22.00 h. However, during the exercise day, 5 samples were collected as follows = 8.00, 12.00, 14.00, 16.00 and 22.00 h.

2.3. Saliva collecting procedure [33]

Step 1 – Do not brush your teeth or eat an hour before sample collection.
Step 2 – Rinse and wash out mouth 3 times with clean tap/bottled water.
Step 3 – Chew a piece of sugar free gum or a piece of straw to stimulate saliva production.
Step 4 – Remove gum/straw from mouth and spit away the first mouthful of saliva to remove excess material from the mouth, this sample is discarded.