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Review article

DHEA: Effects on oxidative and glycative stress and glucose metabolism

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Abstract

An adrenal-derived androgen, dehydroepiandrosterone (DHEA), gradually reduces along with aging. It has been suggested that DHEA is an effective index for aging and has relationships with geriatric syndromes. There are suggestions that the decreased DHEA is related to the onset and progression of metabolic syndrome and diabetes mellitus and the decrease of bone mineral density, in terms of diseases. However, the underlying mechanisms are unknown to a great extent. Focusing on the relationship with diabetes mellitus, a large number of studies on animal models and cells, and cross-sectional studies on humans have suggested that DHEA has anti-diabetic properties. Nevertheless, longitudinal studies on humans and studies on DHEA administration with a small number of subjects have not led to a valid conclusion, providing controversial outcomes. Type II diabetes mellitus is known to exacerbate oxidative stress. The increase of reactive oxygen species in the blood, the decline of defense systems against oxidative stress, and the accumulation of glycation products are observed. Interestingly, it is indicated that DHEA administration to patients with type 2 diabetes improves these phenomena. Inhibitory effects of DHEA on arteriosclerosis are suggested and there is a potential that the effects are exerted along with the above effects against diabetes. DHEA has a variety of favorable properties for hormone replacement therapies in anti-aging fields. Further progress is expected for research on DHEA.

***KEY WORDS*:** dehydroepiandrosterone (DHEA), oxidative stress, glycative stress, glucose metabolism

Introduction

Gradual decline in hormones due to aging is a critical factor that indicates aging process. Gradual decreases due to aging are recognized in growth hormone/insulin-like growth factor-1 system (GH/IGF-1), dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEA-S), and testosterone, which are designated as somatopause, adrenopause, and andropause, respectively[1)], although they are not as drastic as estrogen at menopause. These alternations in hormones are related to aging phenomena such as a subjective loss of sense of well-being and cognitive impairments. Furthermore, the decrease of hormones is considerably related to diverse symptoms that often occur in the middle and old aged, such as obesity, the onset and progression of diabetes and the decline in bone mineral density. From the above perspective, replacement therapies of these hormones are expected to be effective for anti-aging medical treatments. Therefore, studies on hormone replacements have been developed in recent years. The present study, focusing on DHEA, describes the anti-oxidative and anti-glycative actions, which are considered to be mechanisms for anti-aging. DHEA has properties for anti-obesity, anti-diabetes, and anti-arteriosclerosis[1-4)] as has been suggested mainly in research on the cellular level and in animal experiments[1-4)]. The current states of these studies are introduced, placing anti-diabetic effects as the central focus. Furthermore, we introduce a target gene that we identified in DHEA mechanism. At the end of this report, we introduce the meaning of DHEA in sarcopenia from the viewpoint of cortisol/DHEA-S ratio.

What are DHEA and DHEA-S ?

DHEA is an adrenal-derived androgen, and also exists as a sulfate conjugate, DHEA-S in the body. The body can mutually transform DHEA and DHEA-S. They are produced mainly in the adrenal gland and gonad, and some

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are produced in peripheral tissues. DHEA-S is the steroid that exists at the highest concentration in the body. The half-life of DHEA is short at one to three hours and its levels are high in the morning and low in the evening, showing diurnal variations as cortisol levels in the blood do. Contrarily, the half-life of DHEA-S is long at 10 to 20 hours, and does not exhibit as clear diurnal variations as DHEA. However, the alternation of serum DHEA levels in the daytime usually synchronizes to serum DHEA-S levels. Measurement of DHEA-S is common in daily medical practice, as it is easier to measure. While serum cortisol level, which is also an adrenal steroid, does not alter throughout the lifetime, serum DHEA-S levels start to increase at six to seven years of age, reach its peak around 12 to 13 years old, and remain at the highest level until 13 to 25 years old. After that, its levels gradually decrease in a linear fashion along with aging [1)]. In this sense, DHEA-S can be considered a biomarker for aging. Its decline, functionally, is suggested to have a relationship with geriatric syndrome with a functional decline in mental and physical functions due to aging [1-3)].

Deposition of lipofuscin, a lipid peroxide, in adrenal reticularis is observed along with aging. There are diverse theories regarding mechanisms of the decline in DHEA and DHEA-S along with aging. It has been reported that the lipid peroxidation of adrenal microsomes impairs P450c17 activity, which is responsible for DHEA synthesis, and an antioxidant, vitamin E, protects against this impairment [5)]. There is a possibility that the acceleration of adrenal oxidative stress with aging is one of the mechanisms. In fact, recent studies on humans have clarified that the genetic mutations of nicotinamide nucleotide transhydrogenase (NNT) and thioredoxin reductase 2 (TXNRD2), which are anti-oxidative enzymes of mitochondria, induce the onset of adrenal insufficiency [6,7)]. We have observed that ascorbic acid (vitamin C), an anti-oxidative substance, specifically accumulates in adrenal gland [8)]. Also, this suggests the importance of the defense system preventing the adrenal gland from oxidative stress.

Serum DHEA-S concentration level decreases with aging in rhesus monkeys as well as humans. It was reported in an experiment of rhesus monkeys that calorie restriction promoted the extension of lifespan and the group which showed extended longevity had comparatively higher DHEA-S values [9)]. However, afterward, a study reported that calorie restriction did not necessarily change the DHEA-S level in the blood of rhesus monkeys [10)]. Furthermore, it was reported that administration of DHEA did not restore the daily activities of aged rhesus monkeys [11)]. There was a report that six-month calorie restriction on humans did not affect concentration levels of serum DHEA-S [12)]. Further investigations are required to verify the meaning of DHEA-S as a biomarker for longevity assessment under calorie restrictions.

Interestingly, there have been studies regarding longevity, including a research report on inhabitants in Baltimore, U.S.A. Inverse correlations have been identified in male subjects between serum DHEA-S values, and the mortality and the onset of cardiovascular diseases in the follow-up study conducted in Baltimore [9)] and most (not to say all) studies which were conducted on regional inhabitants for long-term periods [13-16)]. In Japan, a retrospective cohort study, which examined inhabitants in Tanushimaru-cho, Fukuoka Prefecture, Japan for 27 years, clarified that survival ratios of males were high in groups with high serum DHEA-S (200 μg/dL or higher) and were the lowest in the group with low serum DHEA-S (lower than 200 μg/dL). No such relation was observed in female subjects [17)]. DHEA is not only a biomarker for aging in both men and women but also a potential biomarker for longevity at least in men.

Mechanisms of DHEA Action

DHEA is a precursor of sex steroids such as estrogens and testosterone. One of the potential mechanisms is the transformation of DHEA into active sex steroids. There is a hypothesis that DHEA exerts its action through a specific mechanism. However, including the existence of its receptor molecules, mechanisms of DHEA action are largely unknown. As another mechanism, DHEA has inhibitory effects against excessive cortisol, which induces neurotoxicity, muscular atrophy, and bone loss. It is advocated that the local glucocorticoid is converted into an inactive form, which is promoted by DHEA via the decrease in 11β-HSD-1 activities and the acceleration of 11β-HSD-2 activities [18,19)]. It is reported that 7α-hydroxy-DHEA, a metabolite of DHEA, is involved in the decrease in 11β-HSD-1 activities due to DHEA [20)].

In sex steroid hormone-sensitive tissues such as bones, prostate and mammary glands, DHEA, which is a steroid precursor for testosterone and estrogen, plays a significant role. DHEA, which is formed in the adrenal gland and the peripheral tissues, is converted to sex steroids in the local tissues of bones, prostate and mammary glands by the so-called intracrinology system. We confirmed that aromatase activity in the osteoblastic cells transformed DHEA into sex steroids and clarified that in fact, the bone mineral density of postmenopausal women had a higher association with serum DHEA-S concentration than with estradiol [21)]. In recent years, CYP17A inhibitor, abiraterone, is an innovative drug for prostatic carcinoma, blocking the provision of androgen from the human adrenal gland and gonad to prostatic carcinoma. The importance of intracrinology has been proven in humans [22)].

It remains unclear regarding the existence of receptors of DHEA(-S) and the mechanism of signal transduction. However, from the viewpoint of outcomes from genome projects, it is considered to be a negative at present that DHEA receptors would exist as nuclear receptors which play a genomic role via genetic transcription. Contrarily, DHEA receptors are firmly suggested to play a non-genomic role as a membrane bound receptor. DHEA(-S) is synthesized as a neurosteroid in the brain. It has been reported mainly in animal experiments that DHEA(-S) enhances memory retention and works as an anti-depressant through neuroprotective and neurotrophic actions. The effects of neurosteroids including DHEA are relatively rapid and receptors in the brain such as NMDA (N-methyl-d-aspartate) receptor, GABA (γ-aminobutyric acid A) receptor and sigma receptors [23)] are reported as molecular targets of DHEA. Although a high degree of affinity of DHEA binding activity has been reported on peripheral blood mononuclear cells [24)] and vascular smooth muscle cells [25)], no conclusive evidence

of the existence of specific receptor molecules has been shown. It is recognized that DHEA has vasodilator actions via nitric oxide (NO)[26]. It was proven that high affinity DHEA receptors, which are a membrane binding type and G protein coupled type, existed (Kd 49pM) and linked with endothelial nitric oxide synthase (eNOS) activation[27].

T lymphocyte cell line, Peer cells, shows high DHEA binding activity under the condition of T cell receptor activation by antigen[28]. We have identified DHEA-induced dual specificity protein phosphatase (DDSP) from the Peer cells[29]. DDSP was induced by DHEA as a target gene of DHEA. DDSP was expressed extensively throughout tissues in the whole body, and phosphatase activities were observed for phosphotyrosine and phosphoserine/threonine, and specific binding to p38 was observed. The MAP kinase system is deeply involved in cell proliferation, canceration, apoptosis, and immune response. There is a possibility that DHEA, through DDSP, exerts diverse physiological activities via inhibitory controls on p38 MAP kinase system (*Fig. 1*). Interestingly, we indicated that in mice with excessive DDSP expression, the weight increase of a male mouse under high-fat diet was significantly inhibited compared with wild type mice[30]. DDSP could explain a part of the anti-obesity mechanism of DHEA. It is assumed that DHEA exerts its physiological activities via multiple mechanisms, such as the action system with membrane receptors of DHEA and its metabolite, the transformation from DHEA to sex steroids, and the counteraction to cortisol.

Anti-atherosclerotic, anti-obesity and anti-diabetic effects of DHEA

(1) Anti-atherosclerotic effects

Studies on anti-atherosclerotic effects of DHEA in humans, both in cross-sectional and longitudinal studies, have suggested that the onset of coronary artery disease is more frequent with lower serum concentrations of DHEA and DHEA-S[14, 16, 31]. At present, there are no studies to verify the effects of DHEA administration on human arteriosclerosis. However, studies with male rabbits indicated that DHEA showed effects on HCD (high cholesterol diet)-induced arteriosclerosis model, where such anti-atherosclerotic DHEA effects were confirmed with repeatability and reproducibility[32-34]. Furthermore, the anti-arteriosclerosis DHEA effects were also observed in research with HCD-induced arteriosclerosis models of Apo E knockout mouse[35]. We identified and reported an anti-atherogenic mechanism in actions of DHEA, clarifying that DHEA markedly inhibited macrophage foam cell formation (cholesterol ester accumulation) induced by modified LDL in cell levels[36]. Interestingly, previous studies suggested that DHEA regulated proinflammatory cytokines and inflammatory signal transductions in immune cells, indicating possibilities of anti-inflammatory actions of DHEA[37], which seems to partly contribute to the anti-atherosclerotic effects.

Oxidative stress is accelerated in type 2 diabetes mellitus (T2DM). The increase of reactive oxygen species

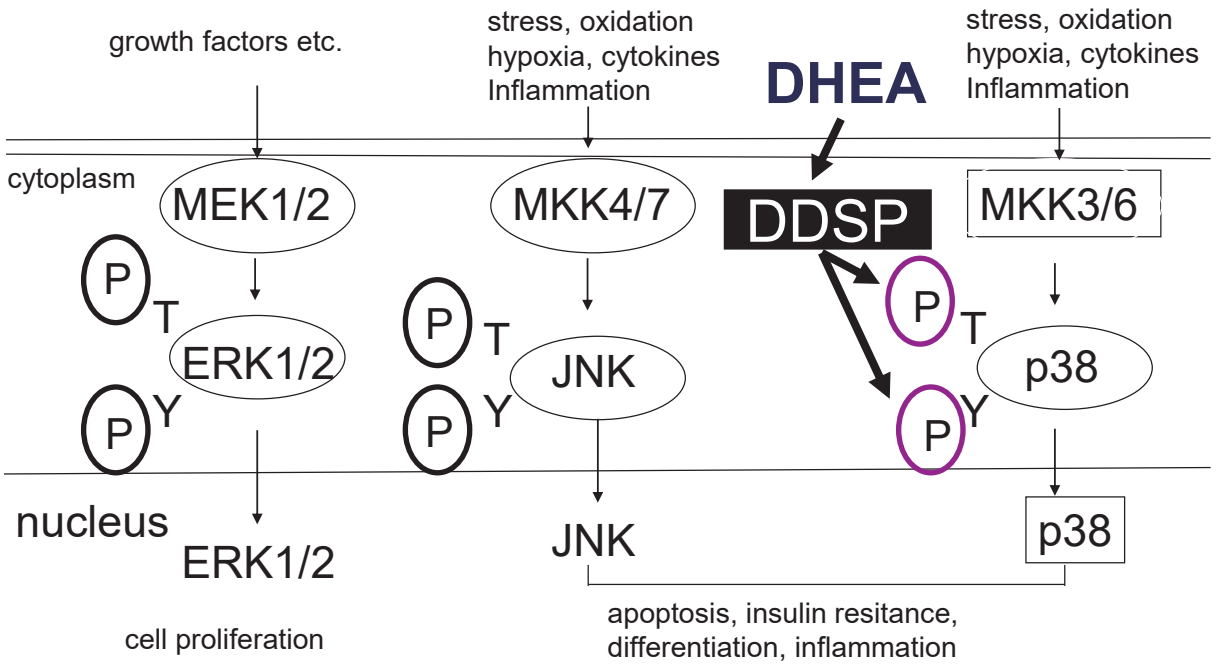

Fig. 1. **Schematic representation of the action of DDSP.**

DDSP is a serine/threonine phosphatase which binds p38 and suppresses its activity. DHEA, dehydroepiandrosterone; DDSP, DHEA-induced dual specificity protein phosphatase. The figure is based on the results of reference 29).

(ROS) in the blood and the decrease of glutathione (GSH) and vitamin E, which are a defense system against oxidative stress, are observed in T2DM patients. An administration of 50 mg DHEA decreased the levels of serum ROS and a biomarker of advanced glycation end products (AGEs), pentosidine, and increased the levels of GSH and vitamin E. That is, DHEA mitigated oxidative stress in T2DM, enhanced the defense system against oxidative stress, and simultaneously, inhibited the accumulation of AGEs (***Fig. 2***)[38]. These findings also suggest another mechanism for anti-atherosclerotic effects of DHEA.

(2) Anti-obesity effects

We reported that DHEA had remarkable anti-obesity effects on Zucker fatty rats, which are rat models with genetic obesity[39]. HCD-induced obesity has been also reported to be improved by DHEA[40]. As possible mechanisms, DHEA is known to increase the level of anti-obesity hormones like estrogen and IGF-1, and increase the fatty acid β-oxidation via activation effects of peroxisome proliferator-activated receptor α (PPARα) in the muscles and the liver[41]. Furthermore, there is a hypothesis that accelerated expression of 11β-HSD-1 in adipose tissues would increase the cortisol production in local fat, and then, induce metabolic syndromes. It was reported that DHEA reduced the cortisol levels in tissues via the modification of adipose cells, 11β-HSD activities[18-20].

A randomized, controlled, double-blind clinical trial in 2004 with a target of 56 subjects at 65-78 years of age (28 men and 28 women) reported the outcome that a significant decrease of visceral and subcutaneous fat and the improvement of insulin resistance were observed in both men and women in groups with DHEA replacement[42]. However, most studies on DHEA administration to humans, excluding this trial, suggested that DHEA effects were neutral in terms of body composition including body weight. For example, in two trial results of influences on body composition including fat, there were no significant differences between groups with DHEA administration and groups without administration of DHEA; one clinical trial which was reported in 2005, providing DHEA administration (50 mg per day) to 140 healthy aged subjects for one year[43], and another in 2006 that administered 50 mg per day to 87 healthy aged female subjects for two years[44].

(3) Effects on skeletal muscles

Research of rat models has suggested that DHEA has the effect of an increase in muscle mass via protein anabolism. That is, DHEA at physiological concentration inhibits the expressions of MuRF-1 mRNA, which is the proteolysis system of rat myoblasts, increases the expressions of myosin heavy chain of muscle contractile proteins, and maintains the muscle mass[45]. In rat strains, exercise and DHEA administration promote *de novo* steroidogenic enzymes in muscles and increases the muscle mass with the increase of testosterone, dihydrotestosterone, and estradiol[46]. There is a positive correlation in humans between the serum DHEA-S concentration and the muscle strength and mass[47]. DHEA

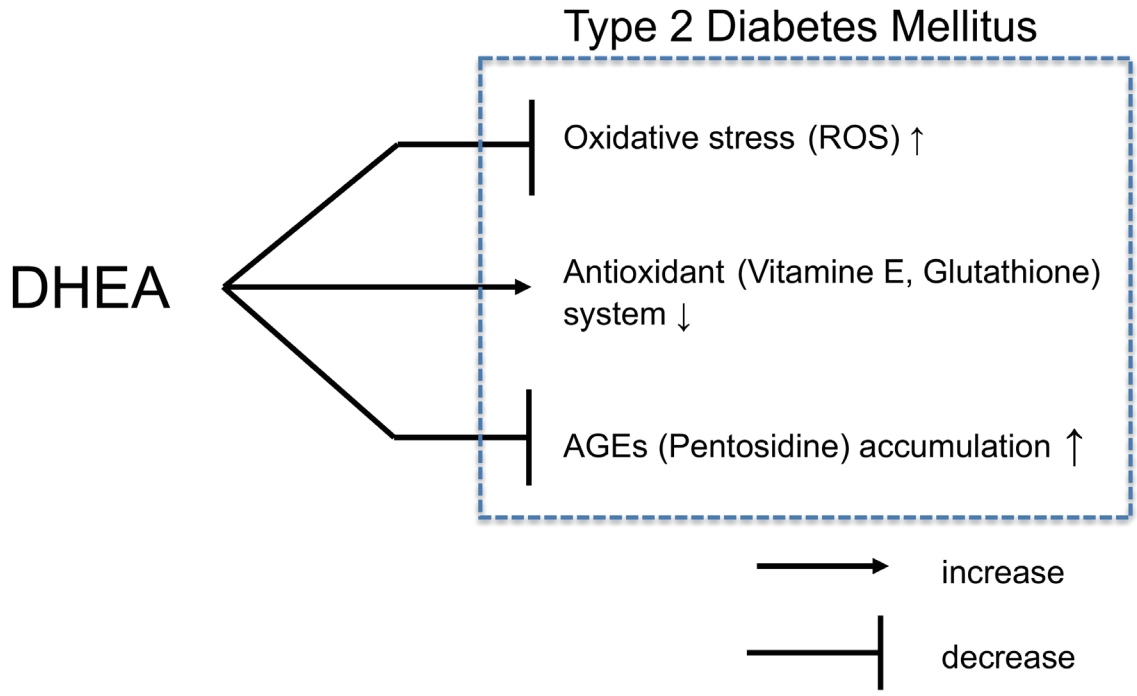

Fig. 2. **Schematic representation of the action of DHEA on oxidative stress, antioxidant system and AGEs accumulation in T2DM.**

DHEA, dehydroepiandrosterone; ROS, reactive oxygen species; AGEs, advanced glycation end products; T2DM, type 2 diabetes mellitus. The figure is based on the results of reference 38).

administration to patients with muscular dystrophy has exhibited an increase in muscle strength and has improved daily activities [48]. For the elderly, exercise and DHEA administration increased muscle strength and mass [49], and the muscle strength of the lower limbs [50]. On the other hand, glucocorticoid including cortisol, shows protein catabolism in most tissues which contain muscles. Glucocorticoid activates ubiquitin-proteasom system in muscles, inducing the degradation of skeletal muscle [51, 52]. We performed a logistic regression analysis on 108 patients with T2DM (age: 65 years old or older, mean age: 76.2 years old) with an objective variable as sarcopenia diagnosed by the Asian version of the diagnostic criteria for sarcopenia. We clarified that the independent risk factor for sarcopenia was an elevation of the serum cortisol/DHEA-S ratio (high value of serum cortisol and low value of serum DHEA-S), in other words, the serum cortisol/DHEA-S ratio ≥ 0.2. The high-level cortisol induces the catabolism of skeletal muscles, while the low-level DHEA reduces the anabolism of skeletal muscle, as is shown in *Fig. 3* [53].

(4) Anti-diabetic effects

Most cross-sectional studies targeting relationships between serum DHEA concentration and diabetes have suggested that the control of diabetes is unfavorable when DHEA levels are lower. However, there are few results of longitudinal studies which suggest significant relations between the onsets of diabetes and serum levels of DHEA or DHEA-S [54-57]. Only one trial, a Rotterdam Study with 5,189 male and female subjects, reported that the incident rate of T2DM was higher when the serum concentration levels of DHEA were lower but there was no association with serum DHEA-S concentration [58]. Results of studies concerning the effect of DHEA administration on insulin resistance in humans were suggested to be controversial; improvement [42, 59], no alternation [44, 60] and deterioration [61]. In particular, a trial regarding DHEA replacement therapy for a relatively long term of two years with a target of 112 elderly subjects, reported that DHEA replacement did not show clear effects in terms of insulin secretion, insulin resistance, and postprandial blood glucose level [60]. A small-scale investigation in Japan reported that oral administration of DHEA to humans (25 mg per day) improved functions of human vascular endothelial cells and insulin resistance [26].

On the other hand, a variety of studies regarding improvement effects of DHEA on insulin resistance and glucose tolerance have been reported, using various types of impaired glucose tolerance models, such as cultured cell models and rodent models. These studies have reported on beneficial glucose tolerance effects with reproducibility [62-67] since Coleman *et al*. reported that DHEA had remarkable effects on db/db mice [62]. Its mechanisms are the inhibition of enzymes responsible for gluconeogenesis like glucose 6-phosphatase (G6Pase) and the enhancing effects of insulin signaling via the activation of the PI3K-AKT system. These studies reported that in cell levels, DHEA had an enhancing effects on glucose uptake via protein kinase C (PKC) in adipose cells, and an enhancing effects on the glucose transportation via glucose transporter 1 and 4 (GLUT1 and 4).

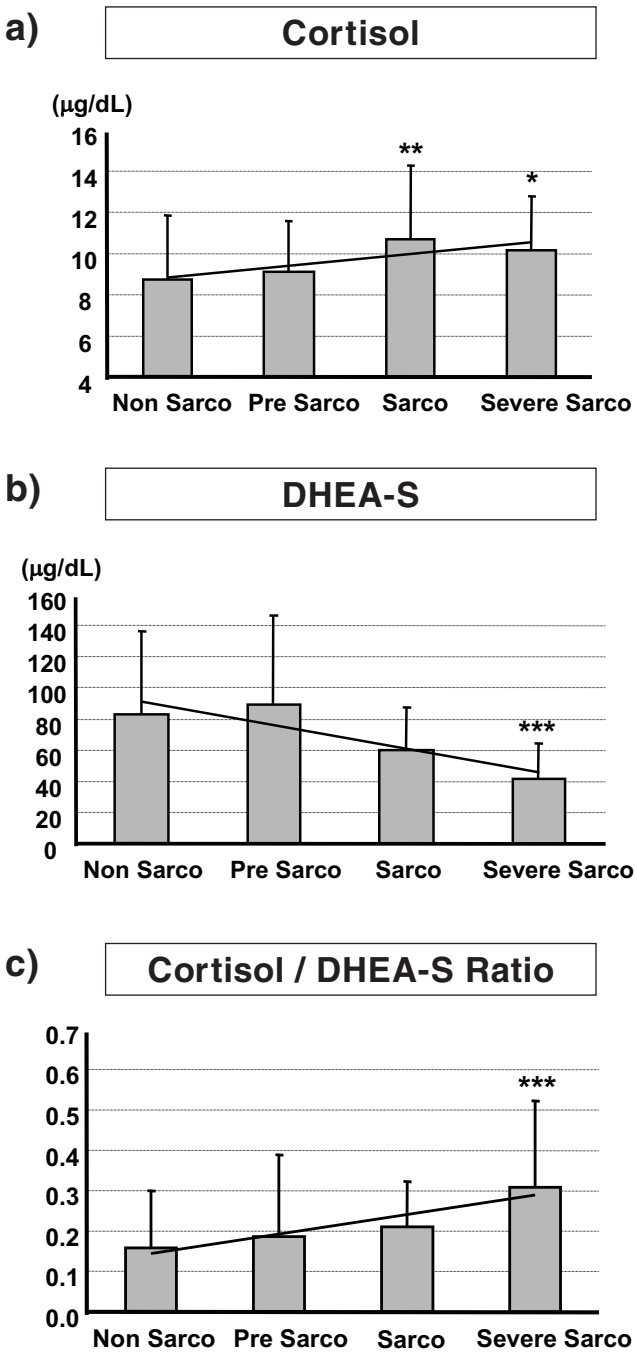

Fig. 3. **Relation between (a) cortisol, (b) DHEA-S, (c) Cortisol/DHEA-S ratio and severity of sarcopenia.**

The graphs are plotted as mean ± SD. *p* values were determined by the Jonckheere–Terpstra test for increased or decreased tendency of continuous variables. **p* < 0.05, ***p* < 0.01, ****p* < 0.001 vs non-sarcopenia (non sarco) as determined by a multiple comparison method (Fisher least significant difference test) after ANOVA.

Pre sarco, presarcopenia; Sarco, sarcopenia; Severe sarco, severe sarcopenia; DHEA-S, dehydroepiandrosterone-sulfate; SD, standard deviation; ANOVA, one wat analysis of variance. The figure is based on the results of reference 53).

Conclusion

Clinical trials on DHEA replacement remain small in number. Clear-cut clinical effects are difficult to observe as long as an administration period of one or two years is concerned. However, few negative aspects such as adverse side effects have been recognized, which is an advantage of DHEA. Further evaluations are required from a long-term perspective. Reductions of serum DHEA-S level are observed in patients with diabetes. However, such reductions can be recovered by the improvement of diabetes control. Thus, voluntary efforts or some medical intervention to improve metabolic and diabetic abnormalities will help persons to raise serum DHEA-S levels and to speed down the slope of DHEA-S decline with aging, leading in the direction of anti-aging.

Conflict of interest

Conflict of interest The author claims no conflict of interest in this study.

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