Dehydroepiandrosterone alters vitamin E status and prevents lipid peroxidation in vitamin E-deficient rats

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In humans, dehydroepiandrosterone and its sulfate ester metabolite DHEA-S are secreted predominantly from the adrenal cortex, and dehydroepiandrosterone is converted to steroid hormones, including androgens and estrogens, and neurosteroid. Dehydroepiandrosterone exerts protective effects against several pathological conditions. Although there are reports on the association between dehydroepiandrosterone and vitamins, the exact relationship between dehydroepiandrosterone and vitamin E remains to be determined. Therefore, we attempted to elucidate the effect of dehydroepiandrosterone on vitamin E status and the expression of various vitamin E-related proteins, including binding proteins, transporters, and cytochrome P450, in vitamin E-deficient rats. Plasma α-tocopherol levels in vitamin E-deficient rats increased in response to dehydroepiandrosterone administration. The expression of hepatic α-tocopherol transfer protein was repressed in vitamin E-deficient rats compared to that in control rats; however, dehydroepiandrosterone administration significantly upregulated this expression. Hepatic expression of CYP4F2, an α-tocopherol metabolizing enzyme, in vitamin E-deficient rats was decreased by dehydroepiandrosterone administration, whereas hepatic expression of ATP-binding cassette transporter A1, an α-tocopherol transporter, was not altered following dehydroepiandrosterone administration. Dehydroepiandrosterone repressed lipid peroxidation in the liver of vitamin E-deficient rats. Therefore, adequate dehydroepiandrosterone supplementation may improve lipid peroxidation under several pathological conditions, and dehydroepiandrosterone may modulate α-tocopherol levels through altered expression of vitamin E-related proteins.

Key Words: dehydroepiandrosterone, α-tocopherol, α-tocopherol transfer protein, lipid peroxidation

Dehydroepiandrosterone (DHEA) and its sulfate ester metabolite DHEA-S are secreted predominantly from the adrenal cortex.[1] DHEA is converted to steroid hormones, including androgens and estrogens, and it plays a critical role in neuronal growth and development as a neurosteroid. DHEA levels gradually increase during adolescence, reaching a maximum in early adulthood, and then decline with age; the levels of DHEA in individuals aged 70–80 years are 10–20% of those present at young adulthood.[1] This decline of DHEA and DHEA-S levels with age is referred to as "adrenopause," and the level of decline differs substantially between individuals. In experimental rodent models, DHEA exerts protective effects against atherosclerosis, cancer, insulin resistance, and obesity. However, human clinical studies on the effect of DHEA administration on the above pathological conditions have yielded conflicting results. Nevertheless, DHEA supplementation for the elderly aged at least 70 years has demonstrated beneficial effects on bone mineral density, skin, muscle strength, and several neuropsychological manifestations, and DHEA can be used as a hormonal replacement therapy for persons of middle or advanced age.

The natural forms of vitamin E include tocopherols and tocotrienols, both of which have are four subtypes, α, β, γ, and δ.[2] All forms of vitamin E have antioxidant properties, including lipoperoxyl radical scavenging activity, and α-tocopherol, which exists mainly in the cell membrane, has the highest antioxidant activity. Vitamin E insufficiency (plasma levels <12 μmol/L) leads to increased rates of infection, anemia, and growth retardation, and has negative effects on both fetus and mother during pregnancy.[3] Inadequate dietary intake of vitamin E causes damage to various target tissues; therefore, intake of 12–15 mg α-tocopherol per day is recommended to maintain good health.

Dietary tocopherols and tocotrienols are absorbed, along with fat, through the intestinal mucosa, and then, vitamin E is packed into chylomicron particles together with lipids, including triglycerol, phospholipid, and cholesterol.[2] Then, the chylomicrons are secreted into the lymphatic and blood circulation, where they are catabolized by lipoprotein lipase. The resulting chylomicron remnants are circulated throughout the body, and subsequently transferred to the liver. In the liver, α-tocopherol is selectively bound to α-tocopherol transfer protein (α-TTP), which is responsible for determining α-tocopherol levels in the body. ATP-binding cassette transporter A1 (ABCA1) plays a critical role in promoting the transport of cholesterol and phospholipid to apolipoproteins and mediating the secretion of α-tocopherol by hepatocytes.[2] α-Tocopherol is incorporated into very low-density lipoprotein (VLDL), and is secreted from the liver into the circulation. Forms of vitamin E other than α-tocopherol have low affinity for α-TTP; therefore, they are promptly catabolized by cytochrome P450. Tocopherols and tocotrienols are metabolized by cytochrome P450 (CYP) 4F2, which initiates their ω-hydroxylation and subsequent oxidation. The metabolites of vitamin E are carboxyethyl hydroxychromans (CEHC), which are sulfated or glucuronidated, and then secreted into the urine and bile.[4] In the circulation, VLDL is hydrolyzed to low-density lipoprotein (LDL) by lipoprotein lipase, and LDL delivers α-tocopherol to peripheral tissues where it is taken up by LDL receptor.[5]

α-TTP is a cytosolic protein that is predominantly expressed in the liver, and is localized to the brain, spleen, lungs, kidneys, intestine, pregnant uterus, placenta, and retina.[5] α-TTP functions to transfer α-tocopherol between membranes. Mice with a disrupted α-TTP gene show markedly lower α-tocopherol levels in

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plasma and tissues and neurological manifestations such as ataxia. α-TTP-deficient mice also show enhanced lipid peroxidation in the brain; therefore, these mice are susceptible to oxidative stress due to vitamin E deficiency.[6] Ataxia with vitamin E deficiency (AVED) is caused by α-TTP gene mutation, which is inherited as an autosomal recessive trait.[7] Patients with AVED have low or undetectable plasma α-tocopherol levels and neurological symptoms similar to Friedreich's ataxia. The phenotype of α-TTP-knockout mice is very similar to the clinical findings of patients with AVED. α-TTP facilitates the release of α-tocopherol from the liver into the circulation.[5]

There are several reports on the effect of DHEA administration on vitamin E status in animals and humans. In retrovirus-infected mice, several symptoms, including cytokine dysregulation, lipid peroxidation, and reduced α-tocopherol levels in the liver, were prevented by DHEA administration,[8,9] and DHEA-S produced identical effects in retrovirus-infected mice.[10] Streptozotocin (STZ)-induced type 1 diabetes model rats present increased reactive oxygen species (ROS) in the brain with hyperglycemia; DHEA treatment of these diabetic rats increased α-tocopherol levels and reduced glutathione levels in brain synaptosomes.[11] In a human study, patients with type 2 diabetes were administered DHEA (50 mg/day) for 12 weeks,[12] and several oxidative biological parameters in plasma and peripheral blood mononuclear cells, including ROS, 4-hydroxynonenal (4-HNE), and glutathione levels, improved. Moreover, plasma vitamin E levels in patients with diabetes, which are lower than those of healthy subjects, increased in response to DHEA supplementation.

Although DHEA administration affects vitamin E status in various pathological conditions, as stated above, the mechanism underlying the regulation of α-tocopherol levels by DHEA remained to be determined. Therefore, in the current study, we investigated the effect of DHEA on vitamin E status and the expression of vitamin E-related proteins, including binding proteins, transporters, and cytochrome P450.

Materials and Methods

Animal experiments. Male 4-week-old Wistar rats and feed were purchased from Japan SLC, Inc. (Sizuoka, Japan) and Funabashi Farm (Chiba, Japan), respectively. Rats were assigned to one of four groups (with five rats per group) and provided free access to the following diets: 1) α-tocopherol (20 mg/kg) in the standard diet for four weeks (control group), 2) standard feed for two weeks and then standard diet containing 0.4% (wt/wt) DHEA for two weeks (DHEA group), 3) α-tocopherol-depleted diet for four weeks (VED group), and 4) α-tocopherol-depleted diet for two weeks and then α-tocopherol-depleted diet containing 0.4% (wt/wt) DHEA for two weeks (VED-DHEA group). DHEA was purchased from Sigma-Aldrich (St. Louis, MO), and diet containing 0.4% (wt/wt) DHEA was previously described.[13] After overnight fasting, the rats were sacrificed by exsanguination under isoflurane anesthesia. Blood was collected in heparinized and non-heparinized tubes, and the plasma and serum were separated and stored at −80°C. The liver tissues were removed, immediately frozen in liquid nitrogen, and stored at −80°C. The care and handling of the experimental animals were performed in accordance with the Osaka Medical College guidelines for the ethical treatment of laboratory animals.

Measurement of lipids, DHEA, 4-HNE, and vitamin E levels. Plasma levels of cholesterol, triglyceride, total l ipids, and alanine aminotransferase (ALT) were determined by enzymatic colorimetric methods. Serum DHEA levels were measured using an ELISA kit (IBL International GmbH, Hamburg, Germany) according to the manufacturer's recommendations. 4-HNE levels in the liver were measured using an OxiSelect HNE-His adduct ELISA kit (Cell Biolabs, San Diego, CA) according to the manufacturer's instructions. Extraction and measurement of plasma and liver α-tocopherol were performed as described previously.[14] α-Tocopherol levels in plasma and liver were adjusted to total lipids and total protein, respectively. Protein content in the liver was measured according to the method of Bradford.[15]

Immunoblottting. Anti-rat CuZn-superoxide dismutase (CuZn-SOD) and Mn-SOD were provided by Dr. Keiichiro Suzuki (Hyogo College of Medicine, Hyogo, Japan), and anti-rat α-TTP IgG was provided by Dr. Hiroyuki Arai (The University of Tokyo, Tokyo, Japan). Rat polyclonal β-actin antibody (Medical & Biological Laboratories Co., Ltd., Nagoya, Japan) was used as a control antibody. Tocopherol-associated protein (TAP)/supernatant protein factor (SPF) was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). The cytosolic fraction of the liver was separated from the liver homogenate by ultracentrifugation at $100,000 \times g$ for 60 min, and protein content was measured using the method of Bradford.[15] The extracted protein was separated by electrophoresis, transferred to a PVDF membrane, and immunoblotted with each primary antibody. Primary antibodies against α-TTP, Mn-SOD, CuZn-SOD, TAP/SPF, and β-actin were used at final dilutions of 1:1,000, 1:10,000, 1:10,000, 1:1,000, and 1:1,000, respectively, in Tris-buffered saline containing Tween-20 (TBS-T), as previously described.[16] Horseradish peroxidase-conjugated goat anti-rat IgG (Bio-Rad Laboratories, Hercules, CA) was used as the secondary antibody, and target bands were detected with the ECL Western Blotting Detection System (GE Healthcare UK Ltd., Buckinghamshire, UK). Relative protein intensities were measured using ImageJ 1.46r software (National Institutes of Health, Bethesda, MD). The ratio of the target protein band intensity to β-actin band intensity (standard) was determined, and the mean and standard deviation of the ratios were calculated.

Cell line and culture. Human hepatocellular carcinoma FLC-5 cells were kindly provided by Dr. Tomokazu Matsuura (Jikei University of Medicine, Tokyo, Japan).[17] FLC-cells were cultivated in ASF104 serum-free medium (Ajinomoto Co., Ltd., Tokyo, Japan), seeded into 6-well plates, and incubated at a constant temperature of 37°C under 5% CO_2/95% air. Cells were passaged using a solution of 25 USP units/ml of trypsin and 0.02% EDTA. At 80% confluence, the spent medium was replaced with fresh medium containing the following ligands or dimethyl sulfoxide (DMSO, vehicle): 1 μM DHEA, 10 μM DHEA, or 50 μM DHEA. The cells were harvested after 24 h, washed twice with phosphate buffered saline, and then used for RNA extraction as described below.

RNA isolation and real-time PCR. Total RNA from rat liver and FLC-5 cells was extracted with ISOGEN (Wako Pure Chem. Ind., Co., Ltd., Osaka, Japan) and the RNeasy Mini kit (Qiagen, Hilden, Germany), respectively. Gene expression in the RNA samples was evaluated by quantitative real-time PCR. First-strand cDNA was synthesized by reverse transcription (RT) using Omniscript (Qiagen). Subsequently, 2 μl of each RT reaction mixture was analyzed by LightCycler PCR (F. Hoffmann-La Roche Ltd. Diagnostics, Basel, Switzerland) using the LightCycler-FastStart DNA Master Hybridization Probe Kit or the FastStart DNA Master SYBR Green I Kit (Roche) according to the manufacturer's protocols. The oligonucleotide primer sequences and accession numbers for target genes are shown in Table 1. Expression of both human and rat α-TTP and β-actin, and rat CYP4F2 were analyzed using the LightCycler-FastStart DNA Master Hybridization Probe Kit, and the expression of rat glutathione peroxidase (GPx) and ABCA1 was analyzed using the FastStart DNA Master SYBR Green I Kit. Expression data for target genes were normalized to β-actin copy number to compensate for differences in RT efficacy among samples as previously described.[18]

Transient transfection and reporter gene assays. Cos7 cells were maintained in DMEM containing 100 units/ml penicillin, 100 μg/ml streptomycin, and 10% fetal bovine serum, and were seeded at a density of 2×10^5/ml in 24-well plates 24 h prior to transfection. Cells were transfected with plasmids in

Table 1. Sequences of primers for real-time PCR

Gene (Accession Number)	Forward	Reverse	Product (bp)
rat ABCA1 (NM_178095)	5'-TTGGGAACGGGTTACTAC-3'	5'-ATGGTCAGCGTGTCACTT-3'	170
rat GPx (NM_030826)	5'-CAATCAGTTCGGACATCA-3'	5'-CGCACTTCTCAAACAATGTA-3'	113
rat α-TTP (NM_013048)	5'-ATTTGATAAATGAGCCGGTC-3' 5'-TAAATCAAGCCTACTTCAGCACTTCCC-3'-FITC LC-5'-GACATTCTTCCTCTGGAATATGGTGGT -3'	5'-TCATTGGATGGTCTCAGAAA-3'	254
rat CYP4F2 (NM_019623)	5'-TGACCCAGATCTACGCTGC-3' LC-5'-GACCCATAAGCCAGTTCCGTTTGGGG-3' 5'-CTCTGTAGGAGTGACCATGCCCACA-3'-FITC	5'-CCACCAACCGAGTCAATTC-3'	141
rat β-actin (V01217 J00691)	5'-CCTGTATGCCTCTGGTCGTA-3' 5'-CGGGACCTGACAGACTACCTCATG-3'-FITC LC-5'-AGATCCTGACCGAGCGTGGCTAC-3'	5'-CCATCTCTTGCTCGAAGTCT-3'	260
human α-TTP (NM_000370)	5'-CTGGCAGCAAAGTTCTTATT-3' 5'-GAGGTAGAAACTCAGCGGAATGGAA-3'-FITC LC-5'-CAACCTTCCAGATCAAAGATAGCCTTG-3'	5'-AATGAATCCGTAAGTACAGCAGC-3'	230
human β-actin (X00351)	5'-CCAACCGCGAGAAGATGAC-3' 5'-CCTCCCCCATGCCATCCTGCGTC-3'-FITC LC-5'-TCAGGTCCCGGCCAGCCAGGTCC-3'	5'-GGAAGGAAGGCTGGAAGAGT-3'	460

Abbreviations: ABCA1, ATP-binding cassette transporter A1; GPx, glutathione peroxidase; α-TTP, α-tocopherol transfer protein; CYP4F2, cytochrome P450 4F2.

serum-free Opti-MEM I medium using Lipofectamine 2000 transfection regent (Life Technologies Corporation, CA) according to the manufacturer's instructions. A thymidine kinase pGL (tk-pGL) luciferase reporter plasmid containing the human α-TTP gene (-1258/-46) was prepared as described previously.[19] Typically, each well contained 2 μl of Lipofectamine 2000, 15 ng of pCMX-hRXRα and pCMX-hSXR expression vectors, 300 ng of pGL-tk luciferase receptor plasmid containing CYP3A4-ER6x3-tk-LUC or the 5'-flanking region of the human α-TTP gene, and 10 ng of CMV-Renilla vector as an internal control for transfection efficacy. The CYP3A4-ER6x3-tk-LUC vector contains the response element for the steroid xenobiotic receptor (SXR)/pregnane X receptor (PXR).[20] After adding reagents, cells were transfected for 6 h at 37°C under 5% CO_2/95% air. The cells were then incubated for 36 h in fresh DMEM containing 5% FBS with 10 μM DHEA, 50 μM DHEA, 100 μM rifampicin, or vehicle. Rifampicin was purchased from Sigma-Aldrich and was dissolved in DMSO. Cells lysates were produced using passive lysis buffer (Promega Corporation, Madison, WI) according to the manufacturer's protocol, and luciferase activity in cell extracts was measured with the Dual Luciferase Reporter Assay System (Promega) using a Luminoskan Ascent (Thermo Fisher Scientific, Waltham, MA).

Statistical analysis. All results are expressed as the mean ± SD and were analyzed by one-way analysis of variance (ANOVA) followed by post hoc analysis using the Tukey-Kramer method. The statistical significance of differences between samples (cell line studies) was examined using Student's t test. A p value less than 0.05 was considered statistically significant.

Results

Biochemical data and α-tocopherol levels. Among the control, DHEA, VED, and VED-DHEA groups, body weight did not differ (Fig. 1A). However, the liver weight and liver/body weight ratio in both DHEA and VED-DHEA rats were higher than those in both control and VED rats (Fig. 1B and C).

In the current study, plasma triglyceride levels among the four groups also did not differ; however, plasma cholesterol levels of DHEA rats were significantly higher than those of control rats (Fig. 2A and B). These elevated cholesterol levels may be due to the function of DHEA as peroxisome proliferator.[21] This finding

is agreement with results we previously reported.[22] However, cholesterol levels did not differ between VED rats and VED-DHEA rats. In addition, ALT levels among the four groups were not different, which shows that neither DEHA supplementation nor vitamin E deficiency led to hepatic dysfunction (Fig. 2C). Serum DHEA levels in both DHEA rats and VED-DHEA rats were significantly higher than those in both control and VED rats (Fig. 2D). The plasma α-tocopherol/lipid ratio also did not differ between control rats and DHEA rats; however, the ratio in VED-DHEA rats was higher than that in VED rats (Fig. 3A). α-Tocopherol levels in the liver of control and VED rats were significantly decreased by DHEA administration (Fig. 3B).

Anti-oxidative enzymes and lipid peroxidation. Expression of antioxidant enzymes, including Mn-SOD, CuZu-SOD, and GPx was not affected by DHEA or vitamin E deficiency (Fig. 4A and B). However, hepatic 4-HNE levels in VED rats were higher than those in control and DHEA rats, and these levels were significantly repressed by DHEA (Fig. 4C).

Expression of α-tocopherol binding proteins in the liver. We assessed the hepatic expression of α-tocopherol binding proteins, including α-TTP and SPF/TAP, in DHEA-supplemented rats. Hepatic α-TTP expression in control rats and DHEA rats did not differ (Fig. 5A). However, α-TTP expression in the liver of VED rats was significantly lower than that of control and DHEA rats, and was markedly increased by DHEA administration. In contrast, hepatic SPF/TAP expression did not differ among the four groups (Fig. 5B).

Expression of α-tocopherol related genes in the liver. Hepatic gene expression of ABCA 1, which encodes an α-tocopherol transporter, did not vary among the control, DHEA, VED, and VED-DHEA groups (Fig. 6A). Expression of CYP4F2, which encodes an α-tocopherol metabolic enzyme, in the liver of both DHEA and VED-DHEA rats was lower than that in both control and VED rats (Fig. 6B). DHEA administration may decrease CYP4F2 gene expression in the liver.

Regulation of α-TTP gene expression. Next, we investigated the transcriptional regulation of α-TTP gene expression. In an *in vitro* assay, the expression of α-TTP mRNA in the hepatic FLC-5 cell line was upregulated by DHEA administration in a dose-dependent manner (Fig. 7A). DHEA is a known ligand of SXR/PXR, which is a member of the nuclear receptor superfamily
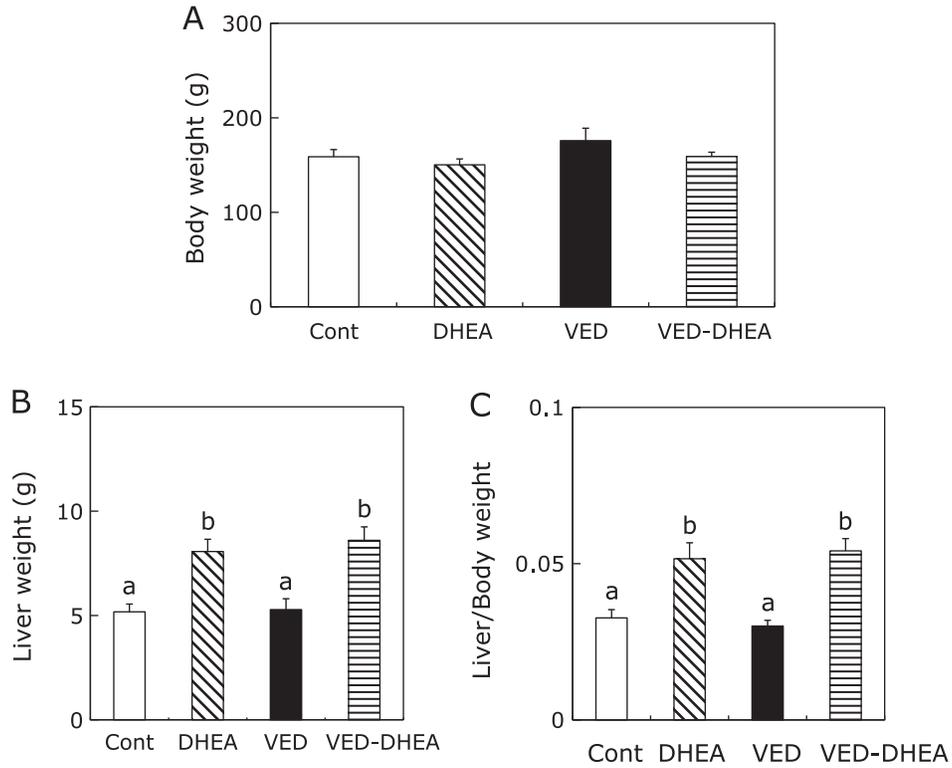
Fig. 1. Body weight, liver weight, and liver/body weight ratio (*n* = 5 per group). Data are presented as means ± SD. Different letters indicate significant differences (*p*<0.05).
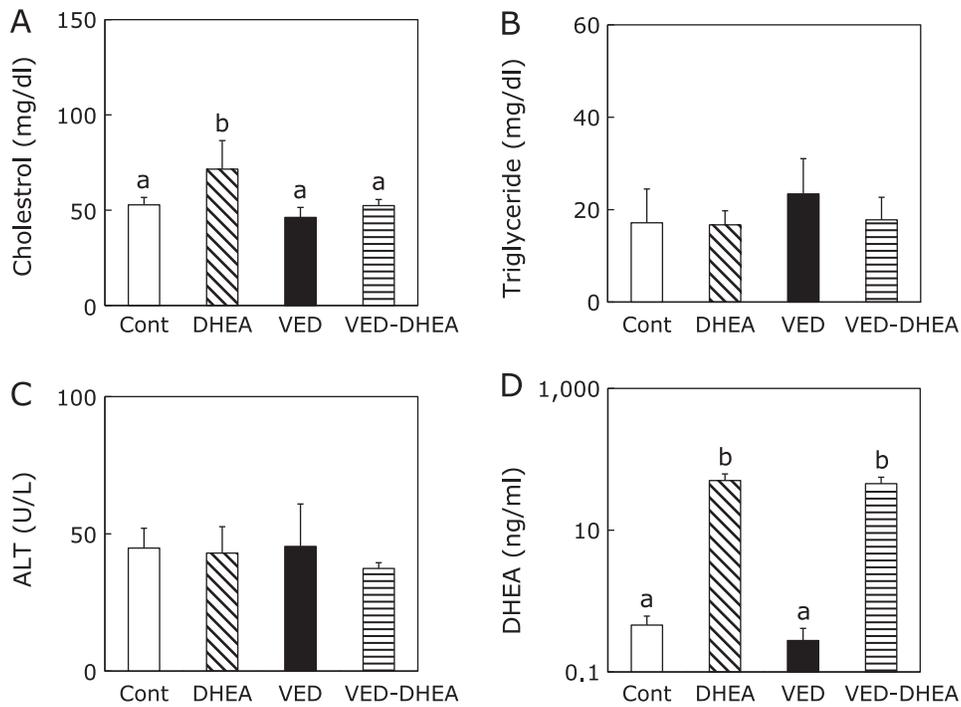
Fig. 2. Plasma lipid and ALT levels and serum DEHA levels (*n* = 5 per group). (A) Cholesterol, (B) triglyceride, (C) ALT, and (D) DHEA levels in each group were measured as described in Materials and Methods. Data are presented as means ± SD. Different letters indicate significant differences (*p*<0.05).
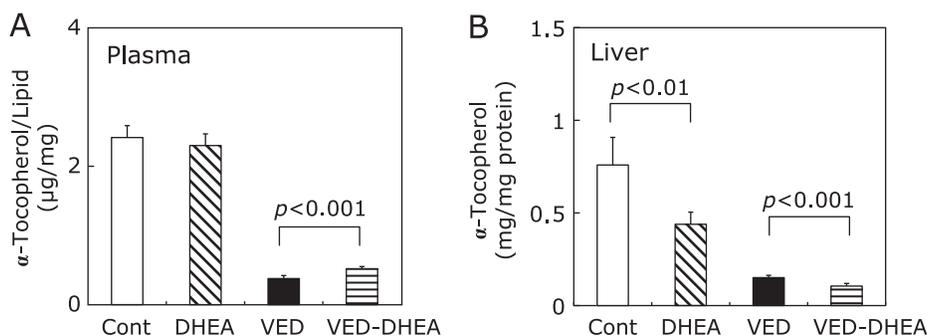
Fig. 3. α-Tocopherol concentrations in plasma and the liver (*n* = 5 per group). The procedure is described in Materials and Methods. Data are presented as means ± SD. Data were analyzed by Student's *t* test, and significant differences are shown in each graph.
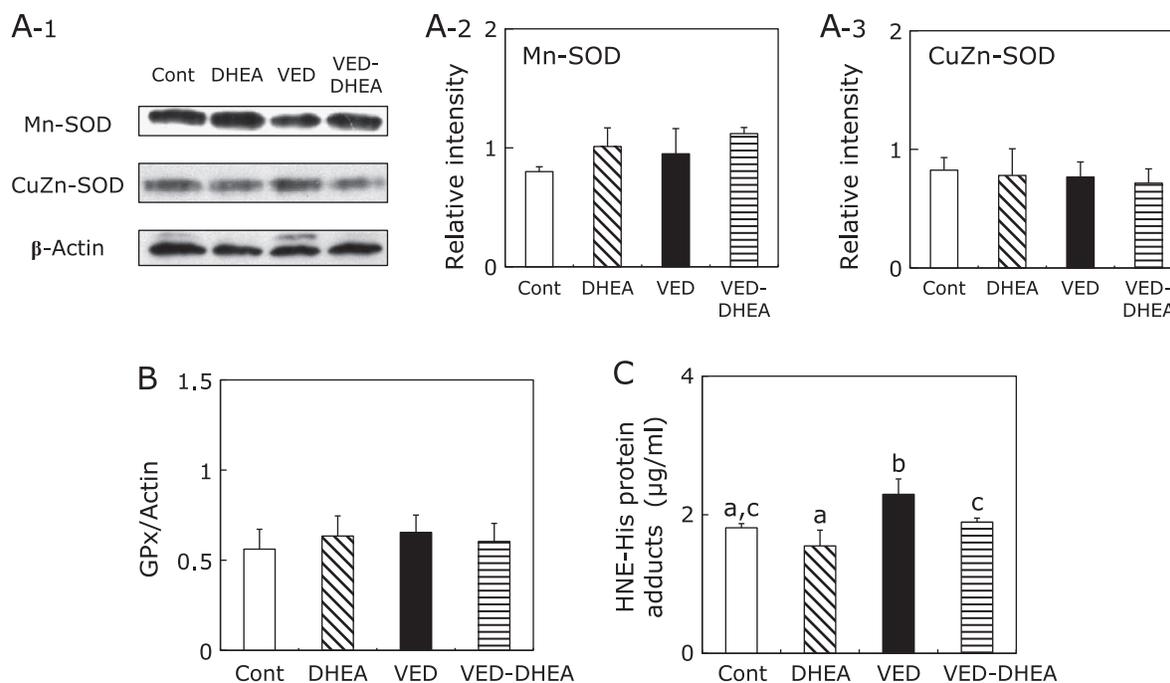
Fig. 4. Expression of Mn-SOD, CuZn-SOD, and GPx in the rat liver, and hepatic HNE-His adduct protein levels. (A) Hepatic Mn-SOD and CuZn-SOD protein expression (*n* = 3 per group) was determined by immunoblotting. (B) The mRNA expression of GPx in the liver (*n* = 5 per group) was assayed by real-time PCR as described in Materials and Methods. (C) Hepatic 4-HNE levels were evaluated by ELISA (*n* = 5 per group). Data are presented as means ± SD. Different letters indicate significant differences (*p*<0.05).

and has xenobiotic functions.[23] We assessed the transcriptional regulation of the human α-TTP expression using a luciferase assay. Luciferase activity was quantified using a luminometer. A luciferase reporter plasmid containing the 5'-flanking region of α-TTP (-1258/-46; approximately 1,300 bp) was co-transfected with human SXR and human retinoid X receptor (RXR)α expression vectors into Cos7 cells (Fig. 7B). The reporter gene containing this region of the human α-TTP gene did not respond to DHEA administration and SXR/RXR. Therefore, in this assay, we did not detect transcriptional regulation of human α-TTP gene by DHEA and SXR/RXR.

Discussion

DHEA, the most abundant adrenal steroid in humans, plays a critical role as a steroidal precursor of sex hormones and has a number of beneficial properties. Although the association between

DHEA and vitamin E has been intensely investigated,[8–12] the relationship between DHEA and vitamin E remains to be determined. Therefore, we elucidated the effect of DHEA administration on vitamin E status and the expression of vitamin E-related proteins. In the present study, plasma α-tocopherol levels in VED rats were increased by DHEA administration. However, α-tocopherol levels in the liver of control and VED rats decreased in response to DHEA administration. This finding may be due to the increased weight of the liver induced by DHEA. In rodent experiments, DHEA acts as a peroxisome proliferation activator, and it induces an increase in liver weight.[21] Body weight did not differ among rats in the four groups, which suggests that their daily feed and dietary α-tocopherol intake may be approximately equal. Therefore, the liver weight gain induced by DHEA administration may be associated with the reduced α-tocopherol levels in the liver.

Evidence suggests that DHEA has beneficial and/or toxic
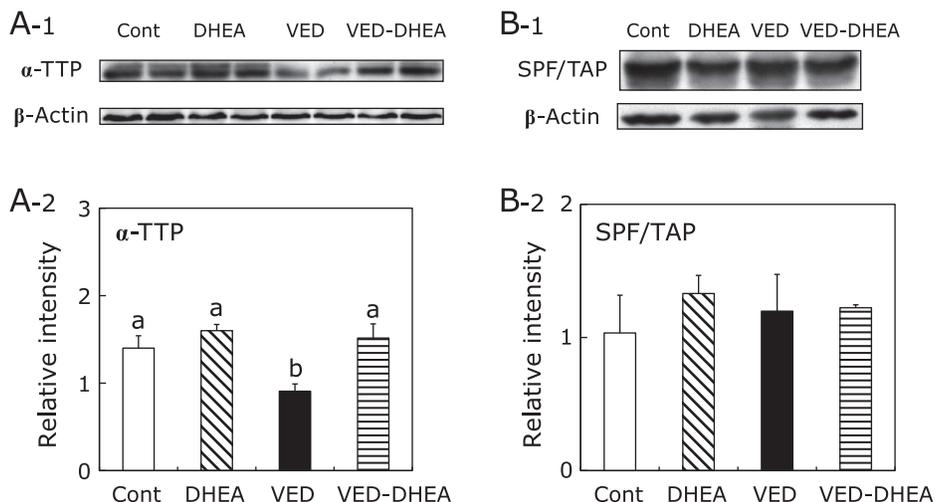
Fig. 5. Expression of α-TTP and SPF/TAP in rat liver (*n* = 3 per group). (A) Hepatic α-TTP and (B) SPF/TAP protein expression was determined by immunoblotting as described in Materials and Methods. Data are presented as means ± SD. Different letters indicate significant differences (*p*<0.05).
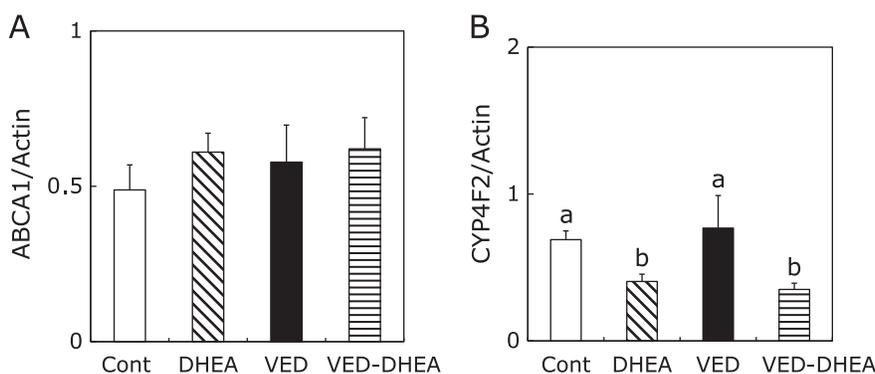
Fig. 6. Expression of ABCA1 and CYP4F2 in rat liver (*n* = 5 per group). (A) Hepatic ABCA1 and (B) CYP4F2 mRNA expression were assayed by real-time PCR as described in Materials and Methods. Data are presented as means ± SD. Different letters indicate significant differences (*p*<0.05).

effects on homeostasis, although it does not act as an antioxidant or a pro-oxidant, but rather alters signaling pathways.[24] There are a number of reports on the preventative effect of DHEA against oxidative stress in several pathological conditions. DHEA administration ameliorated lipid peroxidation induced by hyperglycemia and reduced copper-induced lipid peroxidation of the liver and brain in rats.[25,26] Moreover, DHEA supplementation reduced oxidative stress and improved the antioxidant enzyme activities in the brain of Alzheimer's disease model rats, and improved oxidative stress and renal dysfunction induced by ischemia/reperfusion of rats.[27,28] Gallo *et al.*[29] reported that the opposite effect of DHEA on lipid peroxidation observed *in vitro* is concentration-dependent. DHEA, at levels ranging from 0.1 to 1 μM, prevented lipid peroxidation in liver cells; however, at higher levels (10–50 μM), DHEA promoted toxic actions on cells. In the present study, DHEA administration led to reduced 4-HNE levels in the liver of vitamin E-depleted rats despite the reduced hepatic α-tocopherol levels in VED-DHEA rats. This finding suggests that DHEA exerted antioxidant effects, as mentioned above. The blood DHEA levels of DHEA rats and VED-DHEA rats were approximately 30–60 ng/ml (0.1–0.2 μM). At these levels, DHEA administration protected the liver against lipid peroxidation induced by vitamin E depletion.

In a previous study, lipid peroxidation in the liver of vitamin E-deficient rats was improved by DHEA administration.[30] This finding is in agreement with the results of the present study. Our current findings, as well as those of the above-mentioned animal experiments, show that DHEA acts as antioxidant to prevent lipid peroxidation and modulate α-tocopherol levels in plasma and the liver. These altered circulatory and hepatic α-tocopherol levels might prevent lipid peroxidation. In Ng's study,[30] intraperitoneal injection of DHEA (50 mg/kg or 100 mg/kg, for one week) decreased body weight and elevated the hepatic activities of both glutathione *S*-transferase and glucose-6-phosphate dehydrogenase in rats. However, in the present study, the body weights of rats in the DHEA and VED-DHEA groups did not differ from those of rats in the control and VED groups, respectively. The amount of dietary DHEA fed to DHEA and VED-DHEA rats in the present study was approximately 60–80 mg/day (estimated daily diet: 15–20 g/rat), which is likely similar to the DHEA dosage in Ng's report. The cause for the previously observed difference in body weight is unclear. However, the different routes of DHEA administration (intraperitoneal injection versus oral intake) might lead to differences in circulating DHEA levels and differences in body weight.

DHEA is a modulator of CYP metabolizing enzymes, and it regulates the expression of CYP under various conditions.[31–34] In rodent experiments, DHEA administration enhanced the expres-
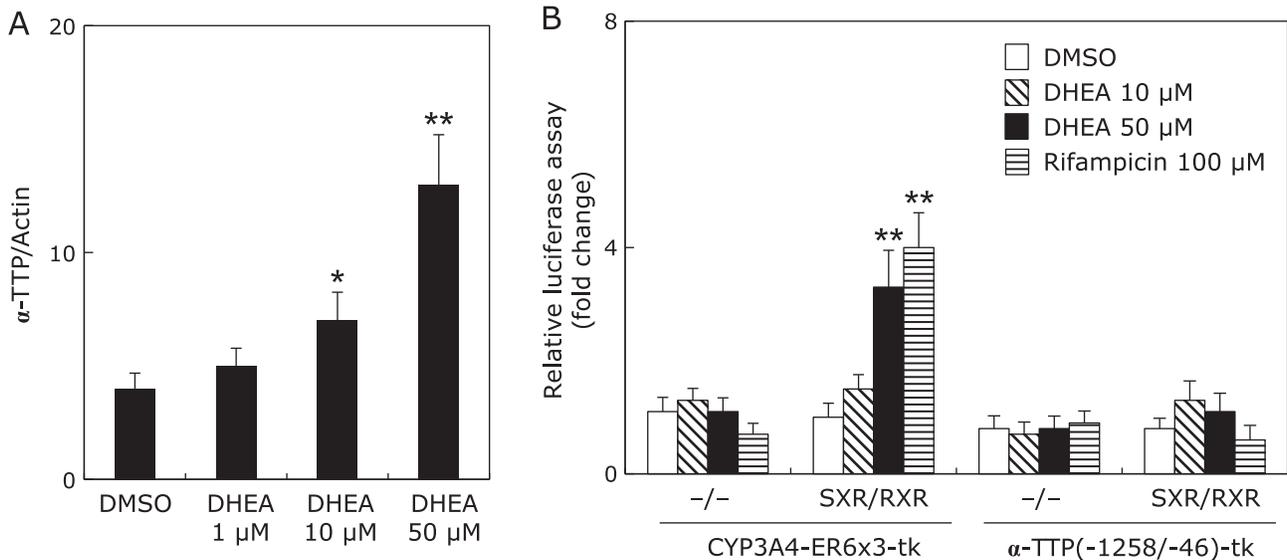
Fig. 7. Effect of DHEA on SXR and RXRα-mediated expression from the human α-TTP promoter. (A) Expression of α-TTP was assessed by real-time PCR. FLC-5 cells were treated with DHEA (1, 10, and 50 μM) as described in Materials and Methods. (B) Cos7 cells were transiently transfected with a human α-TTP promoter vector or a CYP3A4-ER6x3 LUC vector for human SXR and human RXRα and then treated with vehicle (DMSO), 10 μM DHEA, 50 μM DHEA, or 100 μM rifampicin for 36 h before harvest. Luciferase activity in cell lysates was measured using the luciferase assay. Luciferase reporter assays were performed in triplicate, and the data are presented as means ± SD. *$p<0.05$ and **$p<0.001$ compared with vehicle-treated cells, Student's t test.

sion of genes encoding CYP3As, CYP45A, catalase, fatty acyl CoA oxidase, and cytochrome c oxidoreductase.[35] Increased expression of CYP3As and CYP3A23 was mediated via the PXR signaling pathway. In the current study, DHEA administration repressed the expression of hepatic CYP4F2 in rats fed either a normal or a vitamin E-depleted diet. The expression of ABCA1, the cell membrane transporter gene of α-tocopherol, did not differ among the four groups of rats. CYP4F2 catalyzes the ω-hydroxylation of tocopherols and tocotrienols. The decreased expression of CYP4F2 in VED-DHEA rats might be related to the increased plasma α-tocopherol levels because conversion of α-tocopherol to α-CEHC may be reduced. The mechanism underlying the regulation of CYP4F2 expression has not yet been determined; therefore, further investigation will be required to clarify this mechanism.

In the previous studies, the human α-TTP gene was shown to be upregulated by liver X receptor (LXR) and enhanced by peroxisome proliferator-activated receptor (PPAR)α.[19,36] α-TTP expression is modulated by these nuclear receptors to coordinate lipid metabolism. There are several reports on the regulation of hepatic α-TTP expression, with conflicting results regarding α-TTP expression and vitamin E status. Under vitamin E deficiency, α-TTP expression was either increased, decreased, or unchanged.[37–40] Moreover, it was reported that oxidative stress, glucose metabolism, or nutritional status influences hepatic α-TTP expression.[16,36,40–45] Oxidative stress has been shown to have opposing effects on α-TTP expression.[16,36,40–42] In cell line studies, oxidative stress increased α-TTP expression.[36,41] In animal studies, oxidative stress, under highly concentrated oxygen or hepatic dysfunction, induced reduced α-TTP mRNA expression in rats, whereas exposure to environmental tobacco smoke did not affect α-TTP protein expression in mice.[16,40,42] Differences in the experimental conditions, including cell lines, species, and the duration of oxidative stress, might influence α-TTP expression.[19] In the current study, α-TTP expression in VED rats was lower than that in control rats, and the expression was significantly increased by DHEA administration. Lipid peroxidation in the liver of VED rats may affect hepatic α-TTP expression, and the

enhanced expression of α-TTP in VED-DEHA rats may lead to increased plasma α-tocopherol levels. Moreover, the reduced expression of CYP4F2 might also affect α-tocopherol status, as mentioned above. Hence, the enhanced expression of α-TTP accompanied with decreased CYP4F2 expression may increase plasma α-tocopherol levels in VED-DHEA rats. Further experiments are needed to clarify the effect of α-tocopherol-related proteins on α-tocopherol status.

PXR and constitutive androstane receptor (CAR) are the members of a nuclear receptor superfamily that act as xenobiotic receptors.[46] The human homologue of PXR is SXR. Both PXR and CAR regulate the expression of drug transporters and drug metabolizing enzymes that coordinate the absorption, distribution, metabolism, and excretion of chemicals. PXR is a transcriptional factor that regulates the above-mentioned metabolic enzymes, and it is activated by a spectrum of chemical and drug ligands. PXR binds to the promoter of target genes as a heterodimer with RXR on DR3 or DR4 (direct repeats of AG(G/T)TCA or closely related sequences wherein the half-sites are spaced three or four nucleotides apart, respectively), and ER6 (everted repeats spaced six nucleotides apart).[47] DHEA is a ligand of PXR, and it induces the expression of CYP3A.[23] However, in an in vitro assay, DHEA and its metabolites were shown to be relatively weak activators of PXR. In the current study, we analyzed the transcriptional regulation of the human α-TTP gene by SXR and DHEA using a luciferase assay. The human α-TTP promoter used in our study, which contained 1,300 bp of the 5'-flanking region, did not respond to PXR and DHEA, suggesting that the expression of α-TTP is not transcriptionally regulated by PXR and DHEA. However, this experiment has some limitations. First, we used the human α-TTP promoter not the rat promoter, and the regulation of α-TTP gene expression may differ among species. Second, we performed the luciferase assay using approximately 1,300 bp of the 5'-flanking region of the human α-TTP gene, which contains the response element for PPARα and LXR;[19,36] therefore, it may be better to analyze the further upstream of the 5'-flanking region of the human α-TTP gene. DHEA is not a putative endogenous ligand of PPARα; however, it functions as an activator of per-

oxisome proliferation in rodents.[21,48] Therefore, DHEA supplementation in rodents may activate PPAR-mediated target genes in the liver and modulate fatty acid metabolism in the tissue. Moreover, α-TTP is a target gene of PPARα;[36] therefore, α-TTP expression might be increased via PPARα signaling but not via PXR signaling.

In conclusion, DHEA administration altered α-tocopherol status in VED rats, which may be affected by the expression of α-TTP and/or CYP4F2. Moreover, DHEA ameliorated lipid peroxidation in the liver of VED rats. However, there are conflicting findings regarding the antioxidant effect of DHEA in animal and human studies. Adequate DHEA supplementation might improve oxidative stress under several pathological conditions and may modulate α-tocopherol levels through altered expression of vitamin E-related proteins. Further investigation is required to clarify these points.

Acknowledgments

Abbreviations

ABCA1	ATP-binding cassette transporter A1
ALT	alanine aminotransferase
α-TTP	α-tocopherol transfer protein
AVED	ataxia with vitamin E deficiency
CAR	constitutive androstane receptor
CEHC	carboxyethyl hydroxychromans
CYP	cytochrome P450
DHEA	dehydroepiandrosterone
DMSO	dimethyl sulfoxide
GPx	glutathione peroxidase
4-HNE	4-hydroxynonenal
LDL	low-density lipoprotein
LXR	liver X receptor
PPAR	peroxisome proliferator-activated receptor
PXR	pregnane X receptor
ROS	reactive oxigen species
RXR	retinoid X receptor
SOD	superoxide dismutase
SPF	supernatant protein factor
SXR	steroid xenobiotic receptor
TAP	tocopherol associated protein
VED	vitamin E deficiency
VLDL	very low-density lipoprotein

Conflict of Interest

No potential conflicts of interest were disclosed.

References

1 Genazzani AD, Lanzoni C. DHEA as a putative replacement therapy in the elderly. In: Watson RR, ed. *DHEA in Human Health and Aging*. Boca Raton: CRC Press, 2012; 9–34.

2 Jiang Q. Natural forms of vitamin E: metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. *Free Radic Biol Med* 2014; **72**: 76–90.

3 Traber MG. Vitamin E inadequacy in humans: causes and consequences. *Adv Nutr* 2014; **5**: 503–514.

4 Traber MG. Vitamin E regulatory mechanisms. *Annu Rev Nutr* 2007; **27**: 347–362.

5 Kono N, Arai H. Intracellular transport of fat-soluble vitamins A and E. *Traffic* 2015; **16**: 19–34.

6 Yokota T, Igarashi K, Uchihara T, *et al*. Delayed-onset ataxia in mice lacking α-tocopherol transfer protein: model for neuronal degeneration caused by chronic oxidative stress. *Proc Natl Acad Sci USA* 2001; **98**: 15185–15190.

7 Ouahchi K, Arita M, Kayden H, *et al*. Ataxia with isolated vitamin E deficiency is caused by mutations in the α-tocopherol transfer protein. *Nat Genet* 1995; **9**: 141–145.

8 Zhang Z, Araghi-Niknam M, Liang B, *et al*. Prevention of immune dysfunction and vitamin E loss by dehydroepiandrosterone and melatonin supplementation during murine retrovirus infection. *Immunology* 1999; **96**: 291–297.

9 Araghi-Niknam M, Zhang Z, Jiang S, Call O, Eskelson CD, Watson RR. Cytokine dysregulation and increased oxidation is prevented by dehydroepiandrosterone in mice infected with murine leukemia retrovirus. *Proc Soc Exp Biol Med* 1997; **216**: 386–391.

10 Araghi-Niknam M, Ardestani SK, Molitor M, Inserra P, Eskelson CD, Watson RR. Dehydroepiandrosterone (DHEA) sulfate prevents reduction in tissue vitamin E and increased lipid peroxidation due to murine retrovirus infection of aged mice. *Proc Soc Exp Biol Med* 1998; **218**: 210–217.

11 Aragno M, Parola S, Tamagno E, *et al*. Oxidative derangement in rat synaptosomes induced by hyperglycaemia: restorative effect of dehydroepiandrosterone treatment. *Biochem Pharmacol* 2000; **60**: 389–395.

12 Brignardello E, Runzo C, Aragno M, *et al*. Dehydroepiandrosterone administration counteracts oxidative imbalance and advanced glycation end product formation in type 2 diabetic patients. *Diabetes Care* 2007; **30**: 2922–2927.

13 Fujita A, Furutama D, Tanaka T, *et al*. *In vivo* activation of the constitutive androstane receptor β (CARβ) by treatment with dehydroepiandrosterone (DHEA) or DHEA sulfate (DHEA-S). *FEBS Lett* 2002; **532**: 373–378.

14 Tamai H, Manago M, Yokota K, Kitagawa M, Mino M. Determination of α-tocopherol in buccal mucosal cells using an electrochemical detector. *Int J Vitam Nutr Res* 1988; **58**: 202–207.

15 Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; **72**: 248–254.

16 Miyazaki H, Takitani K, Koh M, Yoden A, Tamai H. The α-tocopherol status and expression of α-tocopherol-related proteins in methionine-choline deficient rats treated with vitamin E. *J Clin Biochem Nutr* 2014; **54**: 190–197.

17 Iwahori T, Matsuura T, Maehashi H, *et al*. CYP3A4 inducible model for *in vitro* analysis of human drug metabolism using a bioartificial liver. *Hepatology* 2003; **37**: 665–673.

18 Misaki K, Takitani K, Ogihara T, *et al*. α-Tocopherol content and α-tocopherol transfer protein expression in leukocytes of children with acute leukemia. *Free Radic Res* 2003; **37**: 1037–1042.

19 Koh M, Takitani K, Miyazaki H, Yamaoka S, Tamai H. Liver X receptor up-regulates α-tocopherol transfer protein expression and α-tocopherol status. *J Nutr Biochem* 2013; **24**: 2158–2167.

20 Lehmann JM, McKee DD, Watson MA, Willson TM, Moore JT, Kliewer SA. The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J Clin Invest* 1998; **102**: 1016–1023.

21 Waxman DJ. Role of metabolism in the activation of dehydroepiandrosterone as a peroxisome proliferator. *J Endocrinol* 1996; **150 Suppl**: S129–S147.

22 Takitani K, Miyazaki H, Koh M, Kishi K, Inoue A, Tamai H. Dehydroepiandrosterone alters retinol status and expression of the β-carotene 15, 15'-monooxygenase and lecithin: retinol acyltransferase genes. *J Nutr Sci Vitaminol*; in press.

23 Ripp SL, Fitzpatrick JL, Peters JM, Prough RA. Induction of CYP3A expression by dehydroepiandrosterone: involvement of the pregnane X receptor. *Drug Metab Dispos* 2002; **30**: 570–575.

24 Jacob MH, Araújo AS, Ribeiro MF, Belló-Klein A. DHEA, oxidative stress, and Akt. In: Watson RR, ed. *DHEA in Human Health and Aging* Boca Raton: CRC Press, 2012; 335–339.

25 Aragno M, Brignardello E, Tamagno E, Gatto V, Danni O, Boccuzzi G.

Dehydroepiandrosterone administration prevents the oxidative damage induced by acute hyperglycemia in rats. *J Endocrinol* 1997; **155**: 233–240.

26 Boccuzzi G, Aragno M, Seccia M, *et al*. Protective effect of dehydro-epiandrosterone against copper-induced lipid peroxidation in the rat. *Free Radic Biol Med* 1997; **22**: 1289–1294.

27 Aly HF, Metwally FM, Ahmed HH. Neuroprotective effects of dehydro-epiandrosterone (DHEA) in rat model of Alzheimer's disease. *Acta Biochim Pol* 2011; **58**: 513–520.

28 Aragno M, Cutrin JC, Mastrocola R, *et al*. Oxidative stress and kidney dysfunction due to ischemia/reperfusion in rat: attenuation by dehydro-epiandrosterone. *Kidney Int* 2003; **64**: 836–843.

29 Gallo M, Aragno M, Gatto V, *et al*. Protective effect of dehydroepiandros-terone against lipid peroxidation in a human liver cell line. *Eur J Endocrinol* 1999; **141**: 35–39.

30 Ng HP, Wang YF, Lee CY, Hu ML. Toxicological and antioxidant effects of short-term dehydroepiandrosterone injection in young rats fed diets deficient or adequate in vitamin E. *Food Chem Toxicol* 1999; **37**: 503–508.

31 Webb SJ, Xiao GH, Geoghegan TE, Prough RA. Regulation of CYP4A expression in rat by dehydroepiandrosterone and thyroid hormone. *Mol Pharmacol* 1996; **49**: 276–287.

32 Singleton DW, Lei XD, Webb SJ, Prough RA, Geoghegan TE. Cytochrome P-450 mRNAs are modulated by dehydroepiandrosterone, nafenopin, and triiodothyronine. *Drug Metab Dispos* 1999; **27**: 193–200.

33 Ripp SL, Falkner KC, Pendleton ML, Tamasi V, Prough RA. Regulation of CYP2C11 by dehydroepiandrosterone and peroxisome proliferators: identifi-cation of the negative regulatory region of the gene. *Mol Pharmacol* 2003; **64**: 113–122.

34 Song L, Tang X, Kong Y, Ma H, Zou S. The expression of serum steroid sex hormones and steroidogenic enzymes following intraperitoneal administra-tion of dehydroepiandrosterone (DHEA) in male rats. *Steroids* 2010; **75**: 213–218.

35 Kohalmy K, Tamási V, Kóbori L, *et al*. Dehydroepiandrosterone induces human CYP2B6 through the constitutive androstane receptor. *Drug Metab Dispos* 2007; **35**: 1495–1501.

36 Ulatowski L, Dreussi C, Noy N, Barnholtz-Sloan J, Klein E, Manor D. Expression of the α-tocopherol transfer protein gene is regulated by oxidative stress and common single-nucleotide polymorphisms. *Free Radic Biol Med* 2012; **53**: 2318–2326.

37 Kim HS, Arai H, Arita M, *et al*. Effect of α-tocopherol status on α-tocopherol transfer protein expression and its messenger RNA level in rat liver. *Free Radic Res* 1998; **28**: 87–92.

38 Shaw HM, Huang Cj. Liver α-tocopherol transfer protein and its mRNA are differentially altered by dietary vitamin E deficiency and protein insufficiency in rats. *J Nutr* 1998; **128**: 2348–2354.

39 Fechner H, Schlame M, Guthmann F, Stevens PA, Rüstow B. α- and δ-tocopherol induce expression of hepatic α-tocopherol-transfer-protein mRNA. *Biochem J* 1998; **331**: 577–581.

40 Bella DL, Schock BC, Lim Y, *et al*. Regulation of the α-tocopherol transfer protein in mice: lack of response to dietary vitamin E or oxidative stress. *Lipids* 2006; **41**: 105–112.

41 Etzl RP, Vrekoussis T, Kuhn C, *et al*. Oxidative stress stimulates α-tocopherol transfer protein in human trophoblast tumor cells BeWo. *J Perinat Med* 2012; **40**: 373–378.

42 Ban R, Takitani K, Kim HS, *et al*. α-Tocopherol transfer protein expression in rat liver exposed to hyperoxia. *Free Radic Res* 2002; **36**: 933–938.

43 Miyazaki H, Takitani K, Koh M, Takaya R, Yoden A, Tamai H. α-Tocopherol status and expression of α-tocopherol transfer protein in type 2 diabetic Goto-Kakizaki rats. *J Nutr Sci Vitaminol* 2013; **59**: 64–68.

44 Takitani K, Inoue K, Koh M, *et al*. α-Tocopherol status and altered expres-sion of α-tocopherol-related proteins in streptozotocin- induced type 1 diabetes in rat models. *J Nutr Sci Vitaminol (Tokyo)* 2014; **60**: 380–386.

45 Chen WH, Li YJ, Wang MS, Kang ZC, Huang HL, Shaw HM. Elevation of tissue α-tocopherol levels by conjugated linoleic acid in C57BL/6J mice is not associated with changes in vitamin E absorption or α-carboxyethyl hydroxychroman production. *Nutrition* 2012; **28**: 59–66.

46 Klaassen C, Lu H. Xenobiotic receptors CAR and PXR. In: Bunce CM, Campbell MJ, eds. *Nuclear Receptors*. New York: Springer, 2010; 287–305.

47 Li T, Sonoda J, Evans RM. Establishing orphan nuclear receptors PXR and CAR as xenobiotic receptors. In: Xie W, ed. *Nuclear Receptors in Drug Metabolism*, Hoboken: John Wiley&Sons, 2009; 43–99.

48 Peters JM, Zhou YC, Ram PA, Lee SS, Gonzalez FJ, Waxman DJ. Peroxi-some proliferator-activated receptor alpha required for gene induction by dehydroepiandrosterone-3 beta-sulfate. *Mol Pharmacol* 1996; **50**: 67–74.