Dehydroepiandrosterone Alters Retinol Status and Expression of the β-Carotene 15,15′-Monooxygenase and Lecithin:Retinol Acyltransferase Genes

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Summary

Dehydroepiandrosterone (DHEA) and its sulfate ester DHEA-sulfate (DHEA-S) are the most abundant adrenal steroids in humans. DHEA has a critical role as a steroidal precursor of androgens and/or estrogens, and in human studies and animal experiments, both DHEA and DHEA-S have multiple beneficial effects. However, there are few reports regarding the relationship between DHEA and nutrient status, especially for vitamins. Therefore, we elucidated the effect of DHEA administration on retinol status. Wistar rats were fed with a standard diet containing 0.4% (wt/wt) DHEA for 2 wk. We assessed retinol status and the expression of retinol-related proteins, including metabolic enzymes, binding proteins, cytochrome P450 (CYP) enzymes, and antioxidant enzymes. Retinol levels in the plasma and the liver of DHEA-fed rats were lower than those of controls. Expression of β-carotene 15,15′-monooxygenase (BCMO) in the liver and intestine of DHEA-fed rats was lower, whereas BCMO expression in the testes of DHEA-fed rats was higher than that of controls. Expression of the retinol-metabolizing aldehyde dehydrogenase (ALDH) enzyme ALDH1A2 was repressed in the liver of DHEA rats, whereas ALDH1A1 expression was unaltered. Hepatic expression of lecithin:retinol acyltransferase (LRAT) and CYP26A1 was lower in DHEA-fed rats than in controls. Retinol status in DHEA-fed rats might be affected by altered BCMO expression in the liver and intestine and hepatic LRAT expression, whereas BCMO expression in peripheral tissues may be regulated in a tissue-specific manner. We have shown that DHEA administration may influence retinol status and the expression of retinol-related proteins.

Key Words: dehydroepiandrosterone, retinol, β-carotene 15,15′-monooxygenase, lecithin:retinol acyltransferase

Dehydroepiandrosterone (DHEA) and its sulfoconjugated ester derivative, DHEA-sulfate (DHEA-S), are the most abundant adrenal steroid hormones in humans (1). DHEA levels vary with age, with a peak observed in the early twenties and then a decline in the early thirties (2). DHEA has a critical role as a steroidal precursor of androgens and/or estrogens in peripheral tissues, where DHEA is transformed to sex hormones by steroidogenic and metabolic enzymes (2). In rodent experiments, both DHEA and DHEA-S have beneficial effects on obesity, dyslipidemia, and insulin resistance (3). Human studies demonstrate that DHEA (or DHEA-S) levels are inversely correlated with the incidence of age-associated diseases, including cardiovascular disorders, metabolic diseases, immune dysfunction, cancer, and neurological disorders (1, 3). DHEA administration has a positive effect on bone mineral density (BMD), expressly in post-menopausal women; it has less or no effect on BMD in men (2). Daily DHEA supplementation has anti-diabetic effects in post-menopausal women (2). In animal studies, DHEA inhibited the development of atherosclerosis, whereas DHEA administration had a weak or no effect on this in human clinical trials (4). A number of studies have demonstrated conflicting results and/or discrepancies regarding the effectiveness of DHEA supplementation in human clinical trials (1). The positive effect of DHEA on several pathological conditions remains to be determined. Principally, DHEA administration had no marked adverse side-effects in clinical trials (1).

Retinol, recognized as a fat-soluble nutrient, has essential roles in embryonic development, proliferation and differentiation of cells, immunological function, epithelial barrier function, reproduction, and vision (5). The dietary source of retinol is animal foods, as retinyl esters (predominantly retinyl palmitate), as well as vegetable foods, as carotenoids (mostly β-carotene, α-carotene, and β-cryptoxanthin) (6). Retinyl esters and carotenoids in the diet are absorbed in the upper part of the intestine, where the esterified forms are hydrolyzed. After absorption by the intestinal epithelial cells, a portion of the β-carotene is cleaved to retinal by β-carotene 15,15′-monooxygenase (BCMO), which is the major
β-carotene-metabolizing enzyme and has a critical role in maintaining retinol levels. The major fractions of retinol (recovered as retinyl esters) and carotenoids are packed into chylomicrons, which are released into the lymphocytic circulation. Chylomicrons, containing retinyl esters and carotenoids, are incorporated into the liver, where retinol and β-carotene are metabolized.

Retinol is oxidized to retinal by dehydrogenase, and retinal is sequentially oxidized to retinoic acid by aldehyde dehydrogenase (ALDH). In hepatocytes, retinol and retinal are bound to cellular retinol binding protein (CRBP)-I. Retinoic acid, as the active form of retinol, binds to retinoic acid receptors (RARs) and retinoid X receptors (RXRs), and exerts multifunctional physiological actions in several tissues (7). Retinoic acid (including all-trans retinoic acid and 9-cis retinoic acid) regulates transcription of target genes by serving as a ligand of RARs and RXRs: all-trans retinoic acid serves as a ligand of three subtypes of RAR (RARα, β, and γ), and 9-cis retinoic acid serves as a ligand of the three subtypes of RXR (RXRa, β, and γ). Retinoic acid is catabolized to water-soluble (hydroxy- and oxo-) forms by members of the cytochrome P450 (CYP) enzyme family (CYP26A1, CYP26B1, and CYP26C1) (5). β-Carotene in hepatocytes is cleaved by retinol by BCMO. Retinol in the liver is esterified by lecithin:retinol acyltransferase (LRAT) for storage. When the peripheral tissues need retinol, retinyl esters in the liver are hydrolyzed by retinyl ester hydrolases to retinol, which is bound to retinol binding protein (RBP), and secreted into the circulation (5).

Plasma levels of DHEA and DHEA-S decline with age; therefore these steroid hormones have potential as anti-aging agents and are widely available as anti-aging dietary supplements. However, there are few reports regarding the relationship between DHEA and micronutrient status, including retinol. In a cross-sectional study in healthy men, plasma DHEA levels were positively associated with retinol levels and negatively correlated with fat-soluble α-tocopherol levels (8). In an in vitro study, retinoic acid and retinol increased DHEA levels of ovarian theca cells isolated from healthy women and patients with polycystic ovarian syndrome (9). Moreover, these natural ligands enhanced the expression of the steroidealogenic enzyme cytochrome P450 17α-hydroxylase in ovarian theca cells; this enzyme converts pregnenolone to 17α-hydroxyprogrenolone, and subsequently DHEA. In both in vitro and in vivo studies, all-trans retinoic acid induced sulfotransferase activity in hepatic and intestinal cell lines, and in rat liver and intestine; therefore, DHEA sulfation activity in the cell lines and tissues was enhanced (10). These findings show that retinoic acid and retinol are closely associated with DHEA metabolism. However, there are no reports regarding the influence of DHEA administration on retinol status in human trials or animal experiments. In this study, we examined retinol status and the expression of retinol-related proteins, including metabolic enzymes, binding proteins, and CYP enzymes, in rats administered DHEA.

Table 1. Composition of the diets.

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>8.0%</td>
</tr>
<tr>
<td>Crude protein</td>
<td>20.1%</td>
</tr>
<tr>
<td>Crude fat</td>
<td>4.4%</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>5.2%</td>
</tr>
<tr>
<td>Crude ash</td>
<td>8.8%</td>
</tr>
<tr>
<td>Nitrogen free extracts</td>
<td>5.3%</td>
</tr>
<tr>
<td>Vitamin mixture (containing vitamin A 10.000 IU/kg and β-carotene 0.2 mg/kg)</td>
<td>5.3%</td>
</tr>
<tr>
<td>Total energy</td>
<td>4.09 kcal/g</td>
</tr>
</tbody>
</table>

The diet (MM-3) was purchased from Funabashi Farm (Chiba, Japan).

MATERIALS AND METHODS

Animal experiments. Wistar rats (4 wk old, male) were obtained from Japan SLC, Inc. (Shizuoka, Japan). The care and handling of the experimental animals were performed according to Osaka Medical College guidelines for the ethical treatment of laboratory animals. Feed containing retinol (10,000 IU/kg) and β-carotene (0.2 mg/kg) was purchased from Funabashi Farm (Chiba, Japan) (Table 1). Rats were assigned to two groups (five rats per group), a control group, and a DHEA group fed the standard rat feed containing 0.4% retinol (10,000 IU/kg) and β-carotene (0.2 mg/kg) was purchased from Funabashi Farm (Chiba, Japan). After 2 wk on the control or DHEA-supplemented diet, rats were sacrificed by exsanguination under isoflurane anesthesia after being fasted overnight. Blood was collected into heparinized tubes and plasma was stored at −80°C. The liver, intestine, testes, and kidneys were removed, immediately frozen in liquid nitrogen, and stored at −80°C.

Biochemical data and retinol concentrations. The plasma concentrations of total lipid, cholesterol, triglyceride, and alanine aminotransferase (ALT) were measured by the enzymatic colorimetric method. The retinol concentrations in the plasma and a liver homogenate were assayed by high-performance liquid chromatography, as previously described (12). Liver tissue was homogenized and saponified with a one-twentieth volume of 60% potassium hydroxide in distilled water, the saponified liver samples were extracted with hexane, and the protein content was measured according to the method of Bradford (13). Serum DHEA and DHEA-S levels were measured using an ELISA kit (IBL International GmbH, Hamburg, Germany) according to the manufacturer’s protocols.

Immunoblotting. Anti-CRBP-I (Santa Cruz Biotechnology Inc., Dallas, TX), anti-ALDH1A1 (ProSci Inc., Poway, CA), anti-ALDH1A2 (Santa Cruz Biotechnology Inc.), and anti-β-actin (Medical & Biological Laboratories Co. Ltd., Nagoya, Japan) antibodies were used as primary antibodies. Anti-rat CuZn-superoxide dismutase (CuZn-SOD) and Mn-SOD antibodies were provided by Dr. Keiichiro Suzuki (Hyogo College of Medicine, Nishinomiya, Hyogo, Japan). The cytosolic fraction of the liver
TAKITANI K et al.

Homogenates was analyzed by immunoblotting, and protein content was measured according to the method of Bradford (13). Each primary antibody was appropriately diluted with Tris-buffered saline containing Tween-20, and then used to probe the immunoblot membrane. Each target protein was detected with the appropriate horseradish-peroxidase-conjugated secondary antibody (Bio-Rad Laboratories, Hercules, CA), using the ECL Western Blotting Detection System (GE Healthcare Ltd., UK). Relative protein intensities were determined using the ImageJ 1.46r software (rsb.info.nih.gov/ij). The ratio of the intensity of each cytosolic protein band to that of a /H9252-actin standard was determined, and the mean and standard deviation of these ratios were calculated. An anti-human RBP antibody (Santa Cruz Biotechnology Inc.) was used to assess plasma RBP levels, as previously described (14).

Real-time PCR. Total RNA from individual tissues was purified using ISOGEN (Wako Pure Chemical Industries, Ltd., Osaka, Japan), according to the manufacturer’s instructions. The reverse transcription (RT) reaction was carried out using Omniscript (Qiagen, Valencia, CA), and subsequently each RT reaction mixture was amplified using a LightCycler FastStart DNA Master Hybridization Probe Kit or FastStart DNA Master SYBR Green I Kit (F. Hoffmann-La Roche AG, Basel, Switzerland), according to the manufacturer’s protocol. Quantitative differences were assessed using the ImageJ 1.46r software (rsb.info.nih.gov/ij). The ratio of the intensity of each cytosolic protein band to that of a /H9252-actin standard was determined, and the mean and standard deviation of these ratios were calculated. An anti-human RBP antibody (Santa Cruz Biotechnology Inc.) was used to assess plasma RBP levels, as previously described (14).

Table 2. Sequences of oligonucleotide primers for real-time PCR.

<table>
<thead>
<tr>
<th>Gene (Accession number)</th>
<th>Forward</th>
<th>Reverse</th>
<th>Product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCMO (NM_053648)</td>
<td>5'-CAATGCCTCTTTAAAGTGGT-3'</td>
<td>5'-AAATAACCAGTCAGTCCA-3'</td>
<td>225</td>
</tr>
<tr>
<td>β-Actin (V01217 J00691)</td>
<td>5'-CCCTACAGTCCTGAGTC-3'</td>
<td>5'-CACCTCTTGTGCAATGTC-3'</td>
<td>260</td>
</tr>
<tr>
<td>GPx (NM_030826)</td>
<td>5'-GAATTCGTTGCGATCA-3'</td>
<td>5'-CGCACTCTCAAAACATGTA-3'</td>
<td>113</td>
</tr>
<tr>
<td>CYP26A1 (DQ266888)</td>
<td>5'-GTGGAAGATTGCGGAGA-3'</td>
<td>5'-AGAAGAGATGCGGAGGTC-3'</td>
<td>213</td>
</tr>
<tr>
<td>LRAT (AF255060)</td>
<td>5'-GGAAACCTGAGCAACTG-3'</td>
<td>5'-ACACTAATCCACCGAGA-3'</td>
<td>141</td>
</tr>
</tbody>
</table>

BCMO, β-carotene 15,15′-monooxygenase; GPx, glutathione peroxidase; LRAT, lecithin:retinol acyltransferase.

Table 3. Biochemical data and retinol concentrations.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>DHEA</th>
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<tbody>
<tr>
<td>Total lipid (mg/dL)</td>
<td>312±48.8</td>
<td>355.4±40.2</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>71.2±5.4</td>
<td>105.6±8.7***</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>76.8±23.4</td>
<td>62.2±20.5</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>34±3.8</td>
<td>46.3±13.8</td>
</tr>
<tr>
<td>Retinol (plasma) (µg/mL)</td>
<td>65.4±4.5</td>
<td>40.0±6.6***</td>
</tr>
<tr>
<td>Retinol (liver) (µg/g protein)</td>
<td>0.39±0.07</td>
<td>0.16±0.02**</td>
</tr>
<tr>
<td>DHEA (ng/mL)</td>
<td>0.41±0.17</td>
<td>57.3±8.8**</td>
</tr>
<tr>
<td>DHEA-S (µg/mL)</td>
<td>0.12±0.01</td>
<td>0.8±0.17**</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD and asterisks indicate significant differences (*p<0.05, **p<0.01, ***p<0.001) between control and DHEA-fed rats.

Fig. 1. Plasma retinol binding protein (RBP) expression. Plasma samples from control rats and DHEA-fed rats (n=3 for each group) were analyzed by immunoblotting to detect RBP. Values are expressed as the mean±SD, and significant differences are indicated in the graph.

Fig. 2. Expression of Mn- and CuZn-superoxide dismutase (SOD) proteins, and glutathione peroxidase (GPx) mRNAs in the liver. Liver tissue samples from control rats and DHEA-fed rats (n=3 for each group) were analyzed by immunoblotting to detect the Mn-SOD and CuZn-SOD proteins (panel A, 1–3; n=3 for each group), and by real-time PCR for GPx mRNAs (B; n=5 for each group). Values are expressed as the mean±SD, and significant differences are indicated in the graph.
Titative real-time PCR was performed to determine the levels of mRNAs encoding BCMO, LRAT, glutathione peroxidase (GPx), CYP26A1, and β-actin in total RNA samples, as previously described (15–17). The genes encoding BCMO and β-actin were analyzed using the Master Hybridization Probe Kit, and the Master SYBR Green I Kit was used for analyzing the genes encoding LRAT, GPx, and CYP26A1. The sequences of oligonucleotide primers and accession numbers for the above genes are listed in Table 2. The expression of each gene was adjusted using the β-actin copy number in order to compensate for differences in RT efficiency among samples.

Statistical analysis. Values are expressed as the mean ± SD. To determine the significance of differences, Welch’s t-test was used. Differences between groups were considered significant at a value of \( p < 0.05 \).

RESULTS

Biochemical data and retinol levels

Serum DHEA and DHEA-S levels of DHEA-fed rats were higher than those of controls, which is in agreement with the findings of the previous report (11) (Table 3). In the current study, ALT levels of DHEA-fed rats were not altered compared to those of controls. This result revealed that DHEA administration seemed not to impair hepatic function under the experimental conditions. Total lipid levels and triglyceride levels were not altered between the two groups, but cholesterol levels in DHEA rats were significantly higher than those in the controls. This finding is due to the action of DHEA as a peroxisome activator, particularly in rodents, which may affect lipid profiles (18). The retinol levels in both the plasma and the liver of DHEA-fed rats were lower than those of the controls. Plasma RBP levels of DHEA-fed rats were also low compared to the controls (Fig. 1).

Expression of hepatic antioxidant enzymes

Expression of genes encoding antioxidant enzymes, including Mn-SOD, CuZn-SOD, and GPx, in the liver of DHEA-fed rats did not differ from that of the controls (Fig. 2). Thus, in the present study, DHEA administration did not affect expression of antioxidant enzymes in the liver.

Expression of BCMO in various tissues

The expression of the gene encoding BCMO was examined in peripheral organs, including the liver,
in intestine, kidneys, testes, and lungs (Fig. 3). BCMO gene expression in the liver and intestine of DHEA-fed rats was significantly decreased compared to that of the controls. Conversely, BCMO gene expression in the testes of DHEA-fed rats was higher than that of the controls, whereas that of the lungs and kidneys did not differ between the two groups.

Expression of retinol-related proteins in the liver

The expression of retinol-related proteins, including metabolic enzymes and binding proteins, is shown in Fig. 4 and Fig. 5. For both CRBP-I and ALDH1A1, expression in the liver of DHEA-fed rats did not differ from that in the control group, whereas hepatic ALDH1A2 expression was lower in DHEA-fed rats than in the controls (Fig. 4). Hepatic expression of the genes encoding LRAT and CYP26A1 in DHEA-fed rats was decreased compared to that in controls (Fig. 5).

DISCUSSION

BCMO plays a critical role in maintaining adequate retinol levels, through regulating the conversion of dietary β-carotene to retinal. The gene encoding BCMO in rats is predominantly expressed in the liver and intestine, and weakly in the kidneys, testes, and lungs, which are target organs of retinoic acid as an active metabolite of retinol. The expression pattern of the BCMO gene in the liver and intestine varies in several diseases, including nephrosis, type 1 and type 2 diabetes, obesity with hyperlipidemia, and non-alcoholic fatty liver disease (NAFLD) (14, 16, 17, 19, 20). For instance, type 1 diabetic rats have lower retinol levels in the plasma and liver, and lower BCMO expression in the liver and intestine, than type 2 diabetic rats, which in comparison have higher plasma and hepatic retinol levels, and increased hepatic and intestinal BCMO expression (14, 17). The findings of these previous investigations suggest that expression of the gene encoding BCMO in both the liver and intestine is closely associated with retinol status under pathological conditions. In the present study, BCMO expression in both the liver and intestine of DHEA-fed rats was lower than that of the controls. This lower BCMO expression in DHEA-fed rats might lead to the reduced retinol levels in the plasma and liver. There are no reports regarding the effect of DHEA administration on β-carotene metabolism or absorption in human or animal models. However, the current results regarding retinol metabolism and the expression profiles of retinol-related proteins in DHEA-administered rodent models are not necessarily applicable to humans. Moreover, DHEA in the body is metabolized to DHEA-S and sex hormones (1); therefore DHEA-S and/or sex hormone metabolites might affect retinol status and BCMO expression. Further investigation will be required to clarify this issue.

It is well established that retinol has a critical role in spermatogenesis from the findings of rodent experiments (21). Retinol deficiency or excess in male rats results in the failure of spermatogenesis. RARα−/− gene-disrupted male mice demonstrate azoosperma, and male mice lacking RARβ exhibit spermatic release dysfunction and degenerate testes. Retinoic acid, as well as RARs, is responsible for spermatogonial differentiation (21). In the current study, BCMO expression in the testes of DHEA-fed rats was higher than in controls, which indicated opposite regulation to that in the liver and intestine. In previous studies, we reported that BCMO expression was higher in the testes of streptozotocin-induced type 1 diabetic rats and methionine-choline-deficient NAFLD model rats, both of which had lower plasma retinol levels than the controls (16, 17). These previous results are in agreement with the findings of the current study, and suggest that the testes tissue of DHEA-treated rats might sense the need for retinol due to the reduced plasma retinol levels. Moreover, DHEA-derived androgen metabolites may enhance spermatogenesis, which might also lead to a need for retinol in the testes. Further studies will be required to clarify the mechanism for up-regulation of BCMO expression in rat testes treated with DHEA, which will lead to the definition of the mechanism regulating retinol homeostasis in the testis.

In the current study, expression of the retinol-related metabolizing enzymes ALDH1A2 and CYP26A1 in the liver was repressed by DHEA administration, whereas ALDH1A1 expression was unaltered. Retinoic acid levels are strictly regulated throughout spatiotemporal development of the embryo (22). The ALDH family, including ALDH1A1, ALDH1A2, and ALDH1A3, is responsible for oxidizing retinal to retinoic acid. All ALDH enzymes are expressed in most peripheral tissues.
(23), and expression of each family member is regulated in a different manner, for instance by sex hormones or lipid levels (23). Hence, expression of ALDH1A1 and ALDH1A2 in the liver of DHEA-fed rats may differ. Retinoid acid is catabolized to 4-oxo retinoic acid, 4-hydroxy retinoic acid, and 18-hydroxy retinoic acid by CYP26 family enzymes, including CYP26A1, B1, and C1, which contribute to retinoic acid homeostasis (24). Cyp26a1−/− gene-disrupted mice reveal late embryonic lethality with severe morphological defects. CYP26A1 is predominantly expressed in the liver. The gene encoding CYP26A1 has a retinoic acid response element (RARE) in the promoter region, and its expression is transcriptionally regulated via RAR signaling (24). Therefore, DHEA administration may involve RAR signaling and catabolism of retinol. However, the mechanism by which DHEA influences retinol catabolism remains to be determined. Further experiments are needed to clarify this issue.

From the analysis of LRAT knockout mice, LRAT has been shown to be an essential enzyme for retinol storage in the liver (25). How hepatic retinol levels and LRAT expression are regulated remains to be determined. Generally, hepatic LRAT expression and activity are regulated by dietary retinol and retinoic acid (26); however, the 5 flanking region of the human and rat LRAT genes lack the canonical RARE to which RARs and RXRs bind (27, 28). In animal experiments, hepatic LRAT expression was increased by methionine-choline deficiency in the liver, which was associated with increased plasma retinol levels (20). These findings suggest that altered LRAT expression may be associated with retinol status (16). In the current study, hepatic expression of the gene encoding LRAT is decreased by DHEA administration, which might lead to lowered retinol levels in the liver.

There are some limitations to the present study. Firstly, we used adult rats for this study. Generally, DHEA supplementation is recommended for elderly humans due to age-dependent deficiency of DHEA, and the influence of DHEA on retinol metabolism might be different between adult and elderly rats. Secondly, only male rats were also used in this study. As DHEA is a precursor of steroid sex hormones, the effect of DHEA on retinol metabolism may have yielded different findings in female rats. Thirdly, it is known that the physiological action of DHEA is slightly different in humans and rodents. In rodent experiments, DHEA acts as a peroxisome activator (18), which leads to the involvement of lipid metabolism; this alteration of lipid profiles might affect retinol metabolism. Finally, DHEA is converted to DHEA-S and also some hormone derivatives, which exert the physiological action of DHEA and DHEA-S. These hormones derivatives might affect retinol status; however, we did not elucidate the association of hormones derivatives levels with retinol status.

In the present study, DHEA administration affected the expression of retinol-metabolizing enzymes and retinol status. DHEA is widely consumed as an anti-aging dietary supplement. However, the possibility that at an excessive dose of DHEA may influence retinol status and the expression of retinol-related proteins should be considered. Further investigation is needed to clarify this issue.

Acknowledgments

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Conflict of interest statement

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

REFERENCES


