Long-term administration of DHEA prevents fat deposition in rats fed a high-fat diet

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ABSTRACT: The effects of dehydroepiandrosterone (DHEA) on lipid metabolism and lipogenic gene mRNA expression in rats subjected to a high-fat diet were determined. Totally 75 rats were randomly divided into 5 groups: control group 1 fed a normal diet (NCG), and groups 2–5 fed a high-fat diet with 0 (HCG), 25 (HLG), 50 (HMG), 100 (HHG) mg DHEA per kg body weight via gavage once a day for 8 weeks, respectively. DHEA significantly decreased body weight in HMG group as compared with HCG group (P < 0.05). Hepatic triglyceride and total cholesterol contents were decreased in HMG and HHG groups (P < 0.05), and hepatic lipase activity in HMG group was higher (P < 0.01) than in HCG group. Fatty acid synthesis (FAS) mRNA level was decreased in HLG and HHG groups (P < 0.01), and sterol response element binding protein-1 (SREBP-1) mRNA level was decreased in HMG and HHG groups when compared with HCG group (P < 0.01). Acyl-CoA oxidase (ACO) and liver carnitine palmitoyl transferase-1 (LCPT-1) mRNA abundance was decreased in HLG and HHG groups (P < 0.01), whereas hormone sensitive lipase (HSL) mRNA level was increased in HMG group as compared with HCG group (P < 0.05). These results indicated that long-term administration of DHEA reduced the synthesis of endogenous triglycerides by inhibiting SREBP-1 and FAS expression, and augmented the lipolysis of exogenous triglycerides through enhancing HSL expression, which eventually led to reduced fat deposition in rats fed a high-fat diet.

Keywords: dehydroepiandrosterone; lipid metabolism; lipogenic gene expression

INTRODUCTION

Obesity is the greatest threat to human health (Kopelman 2000; Hernandez-Morante et al. 2011). It is associated with various lifestyle-related diseases, such as type II diabetes mellitus (T2DM), hyperlipidemia, hypertension, and fatty liver disease, which cause a major health burden in terms of morbidity and mortality (Sturm 2007). Dehydroepiandrosterone (DHEA), a naturally occurring steroid, is mainly secreted by the cortex of the adrenal gland. It is a major circulating steroid in humans. DHEA is a very important prohormone, which exerts various physiological activities in rats and mice (Hernandez-Morante et al. 2011). The decrease of DHEA with age is associated with physical health (Labrie et al. 2003). DHEA is commercially available as a non-prescription dietary supplement (Legrain and Girard 2003).

A number of studies in experimental animals and humans have shown that DHEA regulates body adiposity. In rodents, long term DHEA treatment resulted in the suppression of body weight gain without changes in food intake (Cleary et al. 1984). DHEA treatment causes a decrease in metabolic efficiency, and alteration of fat synthesis in rats and mice (Tagliaferro et al. 1995; Hansen et al. 1997; Mauriege et al. 2003; Kochan and Karbowska 2004; Karbowska and Kochan 2005). However, the mechanism of the physiological role of DHEA in lipid metabolism has not yet been well clarified. Moreover, most of previous studies were conducted in rodents fed a regular chow diet (i.e. low fat diet), and its mechanism was illustrated...
mainly through the factors that are involved into endogenous triglyceride synthesis. Information on whether DHEA could prevent the fat deposition in rats under high-fat diet has been sporadic. Oral administration of DHEA, a potential therapy for fat deposition reduction, may be a practical way to reduce excessive fat in human or animal production. Therefore, the present study was carried out to investigate whether a long term administration of DHEA could inhibit fat deposition in rat fed high-fat diet, which may help identify the possible mechanism of DHEA in a decrease of fat deposition in rats or even in humans.

MATERIAL AND METHODS

Animals and dietary treatment. Three-week-old Sprague-Dawley (SD) rats weighing 200 ± 20 g were purchased from the Experimental Animal Center of the Jiangsu University (Nanjing, China). Rats were housed individually in stainless steel wire-bottomed cages at a constant temperature of 25°C and humidity range of 50–60% with a 12-h light/dark cycle. Animals were maintained on standard rodent chow, food and water were available ad libitum for one week. All animal handling procedures were performed in strict accordance with guidelines established by the Institutional Animal Care and Use Committee of Nanjing Agricultural University. After one week of acclimatization, 75 rats were equally randomized into five groups: normal diet control group (NCG), high-fat diet control group (HCG), high-fat diet with low dose group (HLG), high-fat diet with medium dose group (HMG), and high-fat diet with high dose group (HHG). Rats were fed either normal diet (3.57 kcal/g, calories provided by 24.6% protein, 16.4% fat, and 59% carbohydrate; formula GB14924. 3-2010, China) or high-fat diet (4.45 kcal/g, calories provided by 18% protein, 44% fat, and 38% carbohydrate). The NCG group rats were fed normal diet, while the HLG, HMG, HHG groups of rats were fed high-fat diet with DHEA-treatment (purchased from Changzhou Jiaerke Pharmaceuticals Group Corp., and dissolved in 1% DMSO) via gavage at 25, 50, 100 mg/kg body weight respectively once a day for 8 weeks. NCG and HCG group rats received an equal volume of vehicle each day for 8 weeks.

Measurement of serum and liver lipid metabolic parameters. Liver tissues were collected and snap-frozen in liquid nitrogen. Frozen tissues were stored at −70°C before analysis. Blood samples were allowed to clot at 4°C and centrifuged at 3000 g for 10 min before serum harvesting. Prepared serum samples were then stored at −20°C until assayed. The aforementioned blood samples were used to detect blood sugar, total cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL) and to estimate hepatic lipase (HL) activity, all using commercial kits (Nanjing

Table 1. Primer sequence of β-actin and targeted genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>GenBank Acc. No.</th>
<th>Primer sequences (5’–3’)</th>
<th>Orientation</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-actin</td>
<td>NM_031144</td>
<td>CCGTGTGGCTGCTACACGACAGAGGCACCGACGTGGTGACCCCCTGTC</td>
<td>forward/reverse</td>
<td>186</td>
</tr>
<tr>
<td>FAS</td>
<td>NM_017332</td>
<td>GGACATGTCACAGCAGATGACGCAGACAGTGCAGATCCCCTGTC</td>
<td>forward/reverse</td>
<td>94</td>
</tr>
<tr>
<td>SREBP-1</td>
<td>AF286470</td>
<td>GGAGCCATGGATTGCACATTAGGAAGGCTTTCCAGAGAGGA</td>
<td>forward/reverse</td>
<td>194</td>
</tr>
<tr>
<td>ACO</td>
<td>NM017340</td>
<td>CTTTCTTGTGCTTGCTCTCCTTCTTCCGCCTTCACGCCCTCGTA</td>
<td>forward/reverse</td>
<td>415</td>
</tr>
<tr>
<td>ATGL</td>
<td>EU357899</td>
<td>TCACCAACACAGCAGACATCCAAGACATCTCTGGAAGCCACCA</td>
<td>forward/reverse</td>
<td>197</td>
</tr>
<tr>
<td>LCPT-1</td>
<td>NM_031559</td>
<td>AAGAATGGCATCATAGTCCAGTCCAGCTTCTGTCGTG</td>
<td>forward/reverse</td>
<td>203</td>
</tr>
<tr>
<td>HSL</td>
<td>NM_031559</td>
<td>CTCTTTCACTGGCTACTCTCCTTCTCCTGTTTCTGCTTCTG</td>
<td>forward/reverse</td>
<td>192</td>
</tr>
<tr>
<td>PPARα</td>
<td>NM_011144</td>
<td>CTCTGAGCAGAAGAGACACCACGCTGTCACAGAAGCAGCCTTC</td>
<td>forward/reverse</td>
<td>187</td>
</tr>
</tbody>
</table>
Jiancheng Biotechnology Institution, China). Liver TG content was determined using a mixture of chloroform and methanol (2:1 v/v) as described by Folch et al. (1957).

**Real-time PCR.** Total RNA was extracted from liver and adipose samples using TRIZOL reagent (TaKaRa Inc., Dalian, China) according to the manufacturer's protocol. Reverse transcription was performed using the method of Zhao et al. (2007). An aliquot of cDNA sample was mixed with 25 μl SYBR® Green PCR Master Mix (TaKaRa Inc.), in the presence of 10 pmol of each forward and reverse primers for β-actin (used as an internal control), fatty acid synthesis (FAS), sterol response element binding protein-1 (SREBP-1), acyl-CoA oxidase (ACO), peroxisome proliferator-activated receptor-α (PPARα), liver carnitine palmitoyl transferase-1 (LCPT-1) or hormone sensitive lipase (HSL) mRNA (Table 1). All samples were analyzed in duplicate in the ABI Prism 7300 Sequence Detection System (Applied Biosystems, Foster City, USA) and programmed to conduct one cycle (95°C for 1 min) and 40 cycles (95°C for 30 s, 60°C for 30 s, and 72°C for 40 s). Fold change was calculated using the 2−ΔΔCt method. The primers used were designed using the Primer Premier 5 software.

**Statistical analysis.** All statistical procedures, means, and standard errors of the means were computed using SPSS statistical software (Version 17.0, 2008). Data were expressed as means ± SE. Differences were evaluated by two-way ANOVA and were considered significant at \( P < 0.05 \).

## RESULTS

**Effect of DHEA on body weight and epididymal fat.** No obvious differences in body weight were observed in HCG group when compared to NCG group \( (P > 0.05) \). DHEA inhibited body weight gain in the high-fat diet group, and body weight was significantly decreased in the HMG group compared to that in HCG group (Figure 1A). The relative weight of epididymal fat was significantly higher in HCG group \( (P < 0.01) \) than in NCG group (Figure 1B), while it was decreased by 2.72, 5.26, and 1.17% in HLG, HMG, and HHG groups, respectively, when compared with HCG group \( (P > 0.05) \) (Figure 1B).

**Effect of DHEA on lipid parameters in serum.** Compared to NCG group, the TC content was significantly increased in HCG group \( (P < 0.01) \). No significant change was observed in serum glucose and cholesterol contents after administration of DHEA in rats fed high-fat diet when compared with HCG group \( (P > 0.05) \) (Figure 2A,B). However, TG content in HMG group was dramatically decreased \( (P < 0.01) \) when compared with HCG group (Figure 2C), and HDL content was significantly increased in HMG \( (P < 0.01) \) and HHG \( (P < 0.05) \) groups than in HCG group (Figure 2D).

**Effect of DHEA on triglyceride, total cholesterol contents, and hepatic lipase activity in liver.** The TG and TC contents were significantly increased in HCG group than in NCG group \( (P < 0.01) \) (Figure 3). Compared to HCG group, TG content was significantly decreased in HMG \( (P < 0.05) \) and HHG
The TC contents in HMG \((P < 0.01)\) and HHG \((P < 0.05)\) groups were significantly decreased when compared with HCG group. No significant difference was observed in the hepatic lipase activity in HCG when compared with NCG group, while in HMG group it was significantly higher \((P < 0.01)\) than in HCG group.

**Effect of DHEA on hepatic lipid metabolic gene expression.** The \(FAS\) mRNA level (Figure 4A) was significantly increased in HCG than in NCG group \((P < 0.05)\), while no significant differences were observed in \(SREBP-1\) (Figure 4B) mRNA level between HCG group and NCG group \((P > 0.05)\). \(FAS\) mRNA level was markedly decreased in HLG and HHG groups than in HCG group \((P < 0.01)\) (Figure 4A). \(SREBP-1\) mRNA level was significantly inhibited in HMG and HHG groups when compared to HCG group \((P < 0.01)\) (Figure 4B). No significant difference was observed in the \(PPAR\alpha\) mRNA level; \(ACO\) and \(LCPT-1\) mRNA levels were significantly decreased in HCG than in NCG group (Figure 4). The mRNA abundance of \(ACO\), \(LCPT-1\), and \(PPAR\alpha\) was significantly decreased in HLG and HHG groups when compared to HCG group \((P < 0.01)\) (Figure 4C–E).

**Effect of DHEA on the HSL and ATGL mRNA levels in adipose tissue.** Compared to NCG group, hormone sensitive lipase \((HSL)\) mRNA level was significantly decreased \((P < 0.05)\) (Figure 5A), while the adipose triglyceride lipase \((ATGL)\) mRNA level was significantly increased in HCG group \((P < 0.05)\) (Figure 5B). \(ATGL\) mRNA abundance \((P > 0.05)\) was unaffected in the DHEA-treated group when compared with HCG group (Figure 5B), whereas \(HSL\) mRNA level was significantly increased in HMG group than in HCG group \((P < 0.05)\) (Figure 5A).
DISCUSSION

In order to investigate the effect of long-term DHEA administration on fat deposition in rat on a high-fat diet, we first studied the fat deposition in rat fed a high-fat or normal diets. The results showed that rat body weight gain was increased by 2.85% in high-fat diet group than in normal diet group. The relative weight of epididymal fat, hepatic TG and TC contents were significantly increased in rats fed high-fat diet than those in rats fed normal diet. These results indicate that the fat deposition was elevated in rats on a high-fat diet, and this result warrants our further study.

Our results showed that long-term DHEA administration decreased the body weight in rats fed high-fat diet. These results are in line with the previous report that long-term DHEA treatment results in the suppression of body weight gain in rodents under a normal diet (Cleary 1991). Gansler et al. (1985) also reported that long-term DHEA administration to lean or obese Zucker rats resulted in decreased body weight. The relative weight of epididymal fat was reduced by 2.72, 5.26, and 1.17% in rats treated with DHEA when compared with those under a high-fat diet. DHEA presumably reduced the fat deposition, which may lead to the body weight reduction in rats fed a high-fat diet.

DHEA has a fat-reducing effect, however, this effect may be exerted by different mechanisms (De Pergola 2000). The present study showed that hepatic TG and TC contents in rats fed a high-fat diet declined by the administration of 50 and 100 mg DHEA per kg body weight. This observation concurs with the findings of Sabaktarashvili et al. (2005) that DHEA reduces the TC and TG content. In addition, HL is another important lipid metabolic enzyme produced primarily by the liver (Herbst et al. 2003). Our study demonstrated a pronounced increase in HL activity after administration of 50 mg-DHEA per kg body weight in rats fed a high-fat diet. In association with the increased HL activity, there was a decrease in TC content and an increase in HDL content, consistent with the function of HL activity to catalyze the hydrolysis of triacylglycerol and phospholipids.

Figure 3. Effect of DHEA administration on triacylglycerol (A), total cholesterol (B), and hepatic lipase activity (C) in the liver of rats fed a high-fat diet data are expressed as means ± SE, n = 15

**P < 0.01 and *P < 0.05, compared to high-fat diet (HCG) group
and to mediate the removal of lipoproteins from plasma, which resulted in the lipid synthesis decrease. Therefore, DHEA might reduce the fat deposition by reducing the TG content in the adipose tissue in rats under a high-fat diet.

DHEA treatment alters the activities of a lot of enzymes in the liver that are involved in lipid metabolism (Zhao et al. 2007), which could be one of the mechanisms of reducing fat deposition in animal after DHEA administration. The present study showed that FAS mRNA level, the key gene in liver for de novo synthesis of fatty acids, was significantly decreased in the 25 and 50 mg DHEA treatment groups as compared to high-fat diet group. As one of potential regulators, SREBP-1 can directly stimulate the transcription of gene encoding FAS enzymes (Magana et al. 2000), making it a good candidate for controlling lipogenetic genes expression.

Figure 4. Effect of DHEA administration on FAS (A), SREBP-1 (B), ACO (C), LCPT-1 (D), and PPARα (E) genes expression in the liver of rats fed a high-fat diet data are expressed as means ± SE, n = 15

**P < 0.01 and *P < 0.05, compared to high-fat diet (HCG) group
In this study, the \textit{SERBP-1} mRNA expression was significantly suppressed in the 50 and 100 mg/kg DHEA treatment group when compared with that in the high-fat diet group. Based on the above data, one possible explanation for fat deposition decrease after long-term DHEA administration was a dramatical down regulation of transcriptional regulator of \textit{SREBP-1} expression, which altered \textit{FAS} expression that was involved in the fatty acid, inhibiting the synthesis of endogenous TGs. As shown in Figure 4, a significant decrease of \textit{ACO} mRNA level was observed in 50 and 100 mg/kg DHEA treatment groups as compared to the high-fat diet group, which is the first-step enzyme in peroxisomal β-oxidation (Ishii et al. 1985). Our observation showed that \textit{LCPT-1} mRNA level was decreased in 50 and 100 mg/kg DHEA treatment groups, which was accompanied by decreased expression of \textit{ACO}. \textit{LCPT-1} catalyzes the formation of long-chain acylcarnitine from activated fatty acids and free carnitine, thus committing fatty acids to oxidation. By virtue of its inhibition through malonyl-coenzyme A (CoA), \textit{LCPT-1} regulates the synthesis or oxidation of fatty acids (Schmidt et al. 1992). The present study also showed that the \textit{PPARα} mRNA level was decreased in 50 and 100 mg/kg DHEA treatment groups, this was accompanied by the decreased expression of \textit{ACO} and \textit{LCPT-1}. \textit{PPARα} is a member of the nuclear hormone receptor family of transcription factors (Cattley 2003), which regulates the transcription of genes that encode peroxisomal and certain mitochondrial enzymes for fatty acid oxidation (Bremer 2001). Rocchi and Auwerx (2000) have demonstrated that \textit{PPARα} can increase the rate of fatty acid β-oxidation by increasing the expression of several \textit{PPARα} target genes, such as CPT-I and ACO. Together with the decreased abdominal fat caused by DHEA, we presumed that administration of DHEA in rats fed a high-fat diet resulted in significantly decreased \textit{SREBP-1} and \textit{FAS} mRNA levels in liver and thus decreased the rate of fatty acid synthesis in cytosol, which might reversely inhibit the key enzyme involved in β-oxidation of fatty acids and resulting in the decrease of abdominal fat deposition. However, elucidation of the precise mechanism of modifying the gene involved in fatty acid metabolism by DHEA needs further investigation.

The major enzymes involved in the breakdown of TGs in adipose tissue are \textit{ATGL} and \textit{HSL} (Schweigher et al. 2006). \textit{ATGL} is a rate-limiting enzyme of lipolysis that hydrolyzes TG to diglyceride, whereas \textit{HSL} is a multifunctional enzyme that has a broad substrate specificity, with a preference to diglycerides (Lass et al. 2011). It has been demonstrated that TG storage and fatty acid release are mainly influenced by the expression of ATGL in the basal state (Miyoshi et al. 2008). The present results showed that the \textit{ATGL} mRNA level in adipose was significantly increased in HCG group than in NCG group, and the administration of DHEA in rats fed a high-fat diet could inhibit the \textit{ATGL} mRNA expression in adipose as compared to the rats only

Figure 5. Effect of DHEA administration on (A) \textit{HSL} and (B) \textit{ATGL} genes expression in the adipose of rats fed a high-fat diet

data are expressed as means ± SE, \(n = 15\)

\(**P < 0.01\) and \(*P < 0.05\), compared to high-fat diet (HCG) group
under a high-fat diet. Combined with the results of gene expression levels involving in fatty acid metabolism, we presumed that the administration of DHEA could inhibit the synthesis of endogenous TGs which lead to a decrease of the ATGL mRNA level in adipose tissue. Meanwhile the ATGL mRNA level in DHEA treatment groups was obviously higher than in the rat fed normal diet. We have also found that the HSL mRNA level was increased in the DHEA treatment group when compared with the high-fat diet group. These results hinted that the administration of DHEA could increase the ATGL and HSL mRNA levels which accelerated the lipolysis of exogenous TG and reduced the fat deposition in rats under a high-fat diet.

In summary, our findings demonstrate that the long-term administration of DHEA reduced the synthesis of endogenous TGs by inhibiting SREBP-1 and FAS expression and accelerated the lipolysis of exogenous TGs through enhancing ATGL and HSL expression, which eventually reduced fat deposition in the rat fed a high-fat diet.

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