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Short report

The effects of adrenal androgens on glucose metabolism of genetically diabetic mice (*db/db*)

—— differences between dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEA-S) ——

Reiko SAKAI *Department of Nursing, Faculty of Nursing, Aino Gakuin College, Aino Institute for Aging Research*

Daisuke FURUTAMA *Department of Internal Medicine (I), Osaka Medical College*

Tamaki MAEDA *Department of Nursing, Faculty of Nursing and Rehabilitation, Aino University*

Chieko FUJIMURA *Department of Internal Medicine (I), Osaka Medical College*

Toshifumi TANAKA *Department of Nursing, Faculty of Nursing, Aino Gakuin College, Aino Institute for Aging Research*

Toshiaki HANAFUSA *Department of Internal Medicine (I), Osaka Medical College*

Akio KOYAMA *Aino Gakuin*

Nakaaki OHSAWA *Department of Nursing, Faculty of Nursing, Aino Gakuin College, Aino Institute for Aging Research*

Abstract

We investigated the effects of DHEA and DHEA-S on glucose metabolism using diabetic mice *db/db in vivo*. The DHEA-S-treated mice showed more prominent hepatomegaly and a greater decrease of the serum glucose level than DHEA-treated mice. These preliminary data suggest that DHEA-S has a stronger hypoglycemic effect than DHEA, or even that the hypoglycemic effect may be due to DHEA-S, rather than DHEA. The modes of action of DHEA and DHEA-S in diabetic mice should be evaluated from the perspective that these steroids have distinct effects, that can contribute to clarifying the basic pathogenesis of diabetes mellitus.

Keywords: diabetes mellitus, DHEA, DHEA-S, *db/db* mice

Introduction

Dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEA-S) are adrenal androgens, DHEA-S being the most abundant adrenal steroid in humans. Serum levels of DHEA range from 7 to 31 nmol/L, whereas DHEA-S levels range from 2 to 10 μmol/L in men (Orth DN *et al.*, 1992). These androgens show increased serum levels at puberty, reach their highest levels in the second decade of life and decrease with senescence. Based on these changes, there is a physiological significance associated with aging, and possible effects on aging-related diseases have been investigated but there are no reports showing clear data on their effects on aging and aging-related diseases such as diabetes mellitus in humans.

Using rodents, which secrete little or no DHEA and DHEA-S from their adrenal glands, Coleman *et al.* first showed the potential therapeutic value of DHEA on diabetes mellitus (1982). They demonstrated that DHEA causes rapid remission of hyperglycemia and the preservation of islet cells in genetically diabetic mice (*db/db*). Aoki and Sekihara suggested that suppression of elevated activity of hepatic gluconeogenic

enzymes, such as glucose-6-phosphatase, may lead to decreased glucose production in *db/db* mice (1999).

As the metabolism of three steroids and glucose in humans is different from that in mice, the proven effects of DHEA and DHEA-S on hyperglycemia in mice cannot be extrapolated to humans directly. However, investigating the role of these steroids on glucose metabolism *in vivo* is valuable to elucidate the basic pathogenesis of diabetes mellitus.

Previously we observed hepatomegaly with peroxisome proliferation in normal mice treated with DHEA or DHEA-S. The oral administration of DHEA or DHEA-S activated the constitutive androstane receptor ß (CARß) and induced the expression of Cyp2b10, a target gene of CARß, in the liver. However, there was a difference in the degree of Cyp2b10 expression between DHEA-treated mice and DHEA-S-treated mice (Fujita *et al.*, 2002).

Ultimately the mechanism of the effects of DHEA and DHEA-S on hyperglycemia is not completely understood, and the effects of DHEA and DHEA-S have not yet been compared. Here we report the preliminary results of our investigation into differences in the effects of DHEA and DHEA-S *in vivo* using type 2 diabetic (*db/db*) mice.

Materials and methods

Fourteen-week-old male diabetic mice of C 57BL/ksj *db/db* were obtained from CLEA Japan (Tokyo, Japan). Two or three mice were placed in one cage in a temperature-regulated (25±1℃) and humidity-controlled (55%) room with a 12-h light/12-h dark cycle and barrier against pathogens. Water and food (Oriental MF, Oriental, Tokyo, Japan) were available *ad libitum* throughout the experimental period. At 16 weeks of age, the mice were divided into three groups (n=6) and fed with control (standard pellet food), DHEA-containing or DHEA-S-containing diet. DHEA or DHEA-S, obtained from Sigma Chemical Co. (St. Louis, MO, USA), was mixed with standard food powder and pelleted by Oriental Co. Ltd. (Tokyo, Japan) at a final concentration of 0.4% (w/w). The diets were weighed to determine the amount of food eaten each day. Wholebody weights and serum glucose levels of the mice were measured before and after two weeks of feeding. Serum glucose levels were estimated using a portable glucose meter (Glutest, Sanwa Kagaku Kenkyusho Co., Ltd., Nagoya, Japan) in peripheral blood from the tail vein after a one-night fast. On day 16, after glucose measurements were taken, the mice were sacrificed by cervical dislocation and the livers and periepididymal adipose tissues were removed and weighed.

All animal studies were performed after obtaining the approval of Osaka Medical College Animal Care and Use Committee.

Results

Body weights and food intake after the DHEA and DHEA-S treatments are shown in Table 1. The DHEA-treated *db/db* mice ate less than the other groups, but their body weight had not changed significantly by the end of either DHEA or DHEA-S treatment.

The DHEA- and DHEA-S-treated *db/db* mice showed hepatomegaly (Fig. 1A) and their liver weight increased significantly but there was no significant difference in periepididymal adipose tissue after the treatment (Fig. 1B).

The serum glucose level showed a decrease in both the DHEA and DHEA-S groups, and the DHEA-S group showed a greater decrease than DHEA group although there was no significant change between these two groups in day 14 (Fig. 2).

Table 1 The whole body weight and average amount of food eaten each day per head (g) in control, DHEA-treated and DHEA-S treated mice (mean ± SD). Statistical analysis was performed by One-way ANOVA /Turkey's honestly significant difference test. (†: significant)

	whole body weight (g)		
	day 0	day 14	Food intake (g/day)
Control	56.6 ± 2.9	57.3 ± 4.7	5.3 ± 0.3 †
0.4% DHEA	57.0 ± 0.9	57.3 ± 0.4	3.7 ± 0.4 †
0.4% DHEA-S	57.0 ± 1.1	60.1 ± 2.1	4.8 ± 0.6 †

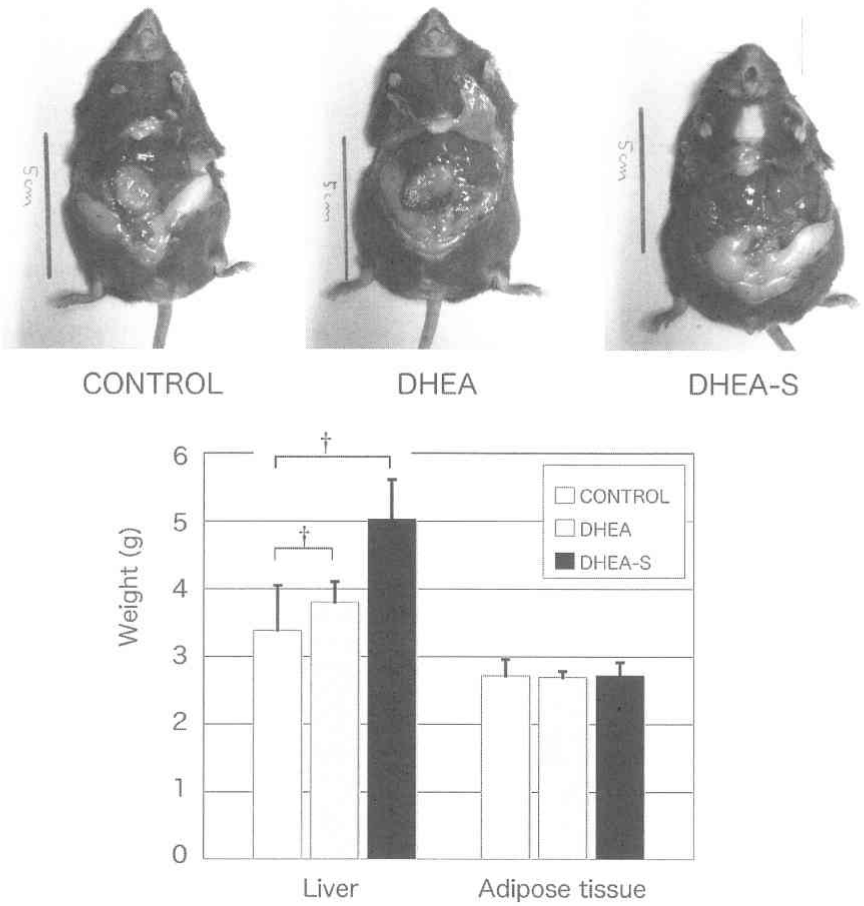

Fig. 1 Change of the liver after the treatments
The autopsy showed the hepatomegaly of the DHEA- and DHEA-S-treated *db/db* mice (A). The liver weights increased in both the treated groups but there was no difference among the periepididymal adipose tissues (B).
Statistical analysis was performed by One-way ANOVA/Tukey's honestly significant difference test (†: significant).

Discussion

Since Coleman *et al.* reported the hypoglycemic effect of DHEA in *db/db* mice (1982), several investigators have reported the mechanisms of DHEA and DHEA-S (Aoki *et al.*, 1999; Dillon JS *et al.*, 2000; Medina MC *et al.*, 2006). However, none of these studies discussed differences in the effects of DHEA and DHEA-S. It is difficult to distinguish their pharmacological mechanisms because DHEA can easily be converted to DHEA-S and *vice versa in vivo*.

To clarify the modes of action, we compared the effects of DHEA and DHEA-S in *db/db* mice and demonstrated differences in liver weights and serum glucose levels between the two groups.

Although the DHEA-treated mice ate relatively less food than the other groups, there was no significant effect on body weight (Table 1). This is likely due to either the relatively brief (two-week) length of the experiment or to differences in metabolism. The greater body-weight gain in DHEA-S-treated mice can be considered a result of liver enlargement (Fig. 1).

Hepatomegaly associated with peroxisome proliferation was also more prominent in DHEA-S-treated normal mice than in DHEA-treated normal mice (Fujita *et al.*, 2002).

As DHEA-S is reported to stimulate peroxisome proliferator activated receptor alpha (PPARα) in the liver (Peters JM *et al.*, 1996), our findings further suggest that hepatomegaly in *db/db* mice may be caused by the action of

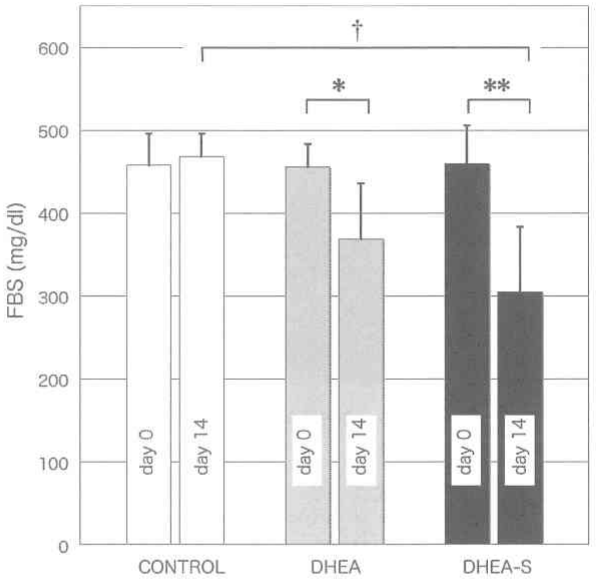

Fig. 2 Serum glucose levels after DHEA or DHEA-S treatments in *db/db* mice.
The serum glucose levels decreased in both the DHEA and DHEA-S mice, but the DHEA-S mice showed a greater decrease.
**: $P<0.01$, *: $P<0.05$: Paired t-test, †: one-way ANOVA/Tukey's honestly significant difference test.

DHEA-S, rather than by DHEA.

In the previous study, we also demonstrated that Cyp2b10 expression showed a correlation with DHEA-S in DHEA-treated mice, but an inverse correlation in DHEA-S-treated mice.

The degree of decrease in serum glucose levels was larger in the DHEA-S-treated group than in the DHEA-treated group (Fig. 2). The concentrations of both DHEA and DHEA-S were elevated in each treatment group, although the amount of DHEA-S found in the DHEA-S-treated group was more than twice the amount found in the DHEA-treated group in normal mice (Fujita *et al.*, 2002). The fact that serum glucose levels decreased in proportion to the concentration of serum DHEA-S suggests that DHEA-S has a stronger hypoglycemic effect than DHEA, or even that the hypoglycemic effect may be due to DHEA-S, rather than DHEA.

We suppose that the pharmacological mechanism might differ between these two chemical structures, and that evaluating differences in their effects in vivo is important to clarify their roles in glucose metabolism.

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