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Dehydroepiandrosterone (DHEA) Ameliorates the Insulin Sensitivity in Older Rats

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To evaluate whether dehydroepiandrosterone (DHEA) ameliorates the decreased insulin sensitivity in older rats, hyperinsulinemic euglycemic clamp studies were performed in rats of various age groups previously treated with DHEA. The glucose metabolic clearance rate (MCR) of the control rats showed a gradual decline with the advancing ages (MCR = 13.05 − 0.027*age (days), r^2=0.683, $p < 0.01$, $n = 18$). The glucose MCR of the DHEA-treated rats also showed a gradual decline with the aging process (MCR = 12.67 − 0.011*age (days), r^2=0.429, $p < 0.01$, $n = 18$). However, the MCR of the DHEA-treated rats were significantly higher than that of control rats. As glucose MCR is a parameter which indicates the insulin sensitivity in various tissues, especially in muscles and body composition, was not changed after the injection of DHEA, DHEA is considered to work on muscles to increase insulin sensitivity. © **1998 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

Dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S) are the most abundant adrenal steroids in man. The metabolism of these hormones is unique among adrenal steroids. The serum concentration of DHEA-S in human plasma is about 300 times higher than that of DHEA and 20 times higher than that of any other steroid hormone. DHEA is considered to be a weak androgen and its concentration has a greater diurnal variation than DHEA-S. Peak serum DHEA and DHEA-S levels occur around age twenties and decrease gradually to 5% of these peak values by the age of ninety. DHEA is synthesized by 17 alpha-hydroxylase of adrenal in human but this enzyme does not express in rat adrenal, so there is little production of DHEA in rat [1]. The physiological importance of DHEA, if any, is still unknown. Yen *et al.* reported an antiobesity effect of DHEA in experimental animals [2]. Since then many other experimental studies, using various kinds of laboratory models, have disclosed a variety of other potential physiological effects of DHEA, including an antidiabetic action. DHEA reduces the hyperglycemia and/or hyperinsulinemia of diabetic mice [3], obese Zucker rats [4] and Sprague–Dawley rats [5] and increases tissue sensitivity of insulin in aged normal mice [6]. The decrease in glucose tolerance with aging has been well evaluated [7–9]. Glucose intolerance develops as a part of the aging process [10]. As only a few experiments have elucidated the mechanism of antidiabetic effect of DHEA in the aged rats, in the present study, we evaluated the effect of exogenous DHEA on tissue sensitivity of insulin especially that associated with aging in rats using hyperinsulinemic euglycemic clamp technique. We found that DHEA increases the sensitivity of insulin in the aged rats. The present paper describes the results.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley (SD) rats, 7–10 week of age, were obtained from Clea Japan (Tokyo), and housed in a temperature- (25°C) and light-controlled (12 h light/day, lights on at 0700 h) room. Food (MF) was purchased from Oriental Yeast (Tokyo).

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Table 1. *Age, body weight and food intake of the control and DHEA-treated rats*

	Numbers	Sex	Age (days)	Body weight (g)	Food intake (g/ body weight/day)
Control	18	male	103.1 ± 30.8*	507 ± 105*	0.040 ± 0.021*
DHEA-treated	18	male	108.6 ± 31.9*	512 ± 102*	0.049 ± 0.018*

NS = not significant; *mean ± SD is shown.

Hyperinsulinemic euglycemic clamp method

Hyperinsulinemic euglycemic clamp studies were performed according to the modified method of DeFronzo *et al.* [11]. The method in brief is as follows. One week after the purchase, four rats were anesthetized with pentobarbital (35 mg/kg i.p.) and catheters (Dow Corning, U.S.A.) were inserted into the jugular vein and carotid artery. Soon after, two rats were injected subcutaneously with DHEA (10 mg/kg body weight) dissolved in 1 ml of sesame oil and another two animals were injected with 1 ml of sesame oil. One week after the operation and the injection of DHEA, euglycemic glucose clamp studies were performed on conscious rats after an overnight fasting. Similar studies using four rats were repeated every week and the total of 36 rats were used for all the studies. The age, body weight and food intake of both groups are shown in Table 1. No significant difference of these values were found between both groups. During the hyperinsulinemic euglycemic clamp, human short acting insulin, Actrapid (Novo Nordisk, Denmark) in 0.9% NaCl solution was infused into jugular vein at 3 mU min^{-1} kg^{-1} with an infusion pump (Terumo, Japan) for 120 min. A 20% glucose solution (Otsuka, Japan) was infused to maintain a blood glucose level of about 6.6 mmol/l. Blood samples were taken from the carotid artery every 10 min and blood glucose levels were monitored by ANTSENSE (Miles-Sankyo, Japan). After the stabliz-ing glucose level, the metabolic clearance rate (MCR) of glucose during the last 30 min was calculated.

Analysis of body composition

10, 20 and 30 weeks of SD rats were used to evaluate the change of body composition. Four rats of each group as controls were decapitated and pretibial muscle, soleus muscle, epididymal fat, retroperitoneal fat and other organs were weighed. One week after the injection of DHEA, DHEA group rats were treated the same as controls.

Statistical analysis

Data were analyzed by Student's *t*-test and linear analysis was carried out by the *F*-test. A *p*-value less than 0.05 was considered statistically significant.

RESULTS

Table 1 shows the age, body weight and food intake of control and DHEA-treated rats. No significant difference of the age, body weight and food intake was found between both groups. The MCR of glucose in the control and DHEA-treated rats at various ages is shown in Fig. 1. In control rats, the MCR of glucose became lower with advancing age. A significant negative correlation was observed between the MCR of glucose and age (MCR = 13.05 − 0.027*age (days), r^2=0.683, $p < 0.01$, $n = 18$). The

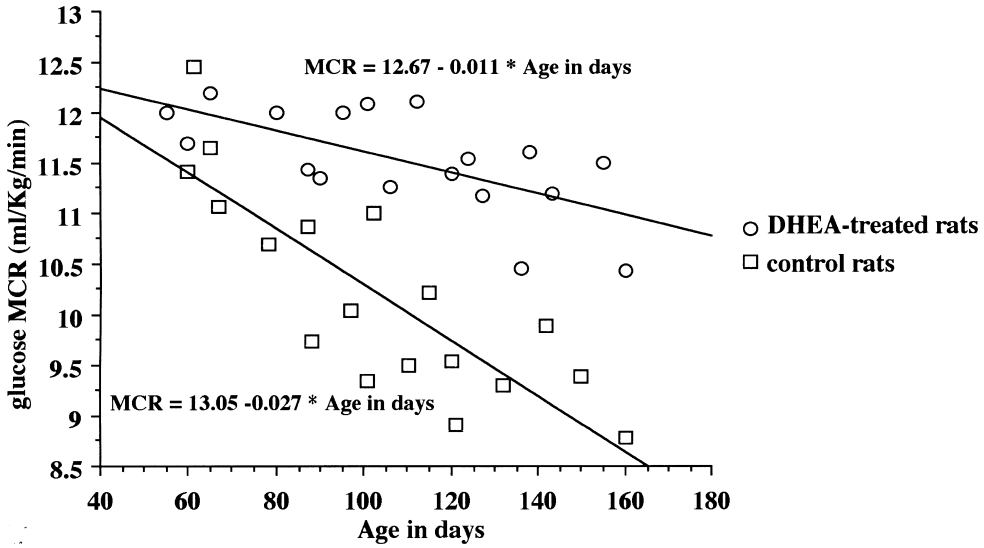

Fig. 1. Glucose metabolic clearance rate (MCR) of the control and DHEA-treated rats of various ages.

glucose MCR of the DHEA-treated rats also showed a gradual decline with advancing age. A significant negative correlation was also found between glucose MCR and age (MCR = 12.67 − 0.011*age (days), r^2=0.429, $p < 0.01$, $n = 18$). The K value of the curves for the DHEA-treated rats was significantly higher than that for control rats ($p < 0.05$). The change of the body composition of DHEA-treated rats is shown in Fig. 2. No significant difference was found between control and DHEA-treated rats.

DISCUSSION

Several studies have shown that DHEA exerts an antidiabetic effect. Coleman *et al.* reported that following DHEA treatment, C57 BL/KsJ db/db mice have improved glucose tolerance in comparison to non-treated mice [3]. Pashko *et al.* reported that the oral administration of a DHEA analogue which lacks the androgenic action, reduces serum glucose levels in C57BL/KsJ db/db mice [12]. To elucidate further the mechanisms of this antidiabetic effect of DHEA, we evaluated the effect of DHEA on the MCR of glucose in rats using a glucose clamp technique to determine whether the effect of DHEA is caused by ameliorating insulin resistance. As the MCR of glucose has been reported to decrease with advancing ages in rats [13], we performed these studies using older rats in addition to younger animals. The results of the present study demonstrated that DHEA ameliorates the decline of MCR of older rats although it shows little change in the younger rats. The MCR of glucose obtained from hyperinsulinemic euglycemic clamp technique is considered to show the insulin sensitivity in muscles. As the muscle volume did not increase in the DHEA-treated rats, the increase in glucose MCR is considered to indicate the amelioration of insulin sensitivity in muscles. Therefore, DHEA may act on muscular cells and ameliorate insulin sensitivity. Although in rats little DHEA is secreted from the adrenal cortex, DHEA is secreted from the adrenal cortex in man, indicating that DHEA is an endogenous insulin sensitizer. Further details of the mechanism of the insulin sensitizing effect of DHEA in muscles should be evaluated in the following studies.

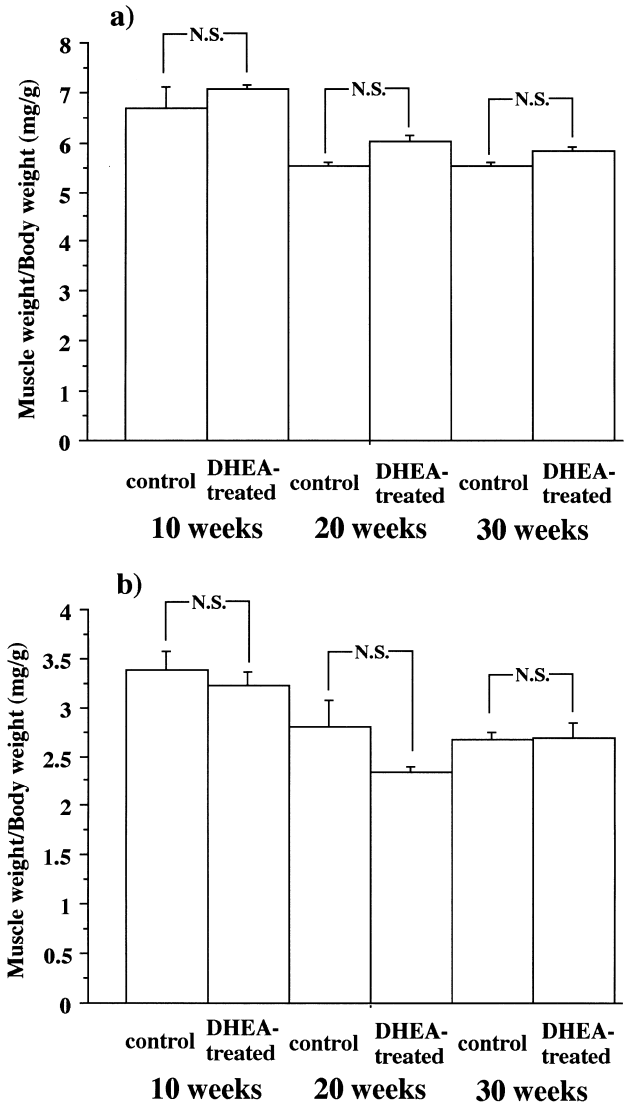

Fig. 2. (a) Ratio of soleus muscle weight to body weight. (b) Ratio of pretibial muscle weight to body weight. Each bilateral (right and left) muscle were averaged.

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