RAPID COMMUNICATION

Dehydroepiandrosterone Suppresses Elevated Hepatic Glucose-6-phosphatase mRNA Level in C57BL/KsJ-db/db Mice: Comparison with Troglitazone

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Abstract. Dehydroepiandrosterone (DHEA) is known to improve hyperglycemia of diabetic C57BL/KsJ-db/db mice that are obese and insulin resistant. In a previous study, we reported that DHEA as well as troglitazone suppresses the elevated hepatic gluconeogenic enzymes, glucose-6-phosphatase (G6Pase) and fructose-1,6-bisphosphatase (FBPase) activities in C57BL/KsJ-db/db mice. In the present study, we evaluated the changes in mRNA of G6Pase and FBPase in db/db mice. Despite hyperinsulinemia, the G6Pase mRNA level of db/db mice was elevated as compared to their heterozygote littermate db/+m mice. In contrast, the FBPase mRNA level was not elevated in db/db mice. Administration of DHEA for two weeks significantly decreased the blood glucose level and the elevated G6Pase mRNA level in db/db mice. No significant changes were seen in the FBPase mRNA level after the administration of DHEA. Administration of troglitazone also decreased the blood glucose and G6Pase mRNA level in db/db mice although no changes were seen in the FBPase mRNA level. These results suggest that the elevation of G6Pase mRNA is important in elucidating the cause of insulin resistance, and that the G6Pase gene is at least one target for the hypoglycemic effects of DHEA as an insulin sensitizing agent in db/db mice.

Key words: DHEA, Troglitazone, db/db Mouse, Glucose-6-phosphatase

C57BL/KsJ-db/db mice have been shown to become obese, hyperglycemic, and hyperinsulinemic [1]. Coleman et al. reported that dietary administration of dehydroepiandrosterone (DHEA), a major adrenal androgen, to the db/db mice induced remission of hyperglycemia and increased insulin sensitivity [2, 3]. Cleary, and McIntosh and Berdanier indicated the antiobesity effects of DHEA in rats [4, 5], and Richards et al. and Abadie et al. reported that DHEA altered lipids in diabetic rats [6, 7]. Many studies suggest that DHEA increases the sensitivity of insulin [8-10]. Troglitazone is the first hypoglycemic agent to be introduced into clinical medicine to treat diabetic patients that are insulin resistant.

In a previous study [11], we evaluated the activities of hepatic gluconeogenic enzymes to clarify the mechanism of the insulin sensitizing effect of DHEA. Glucose-6-phosphatase (G6Pase) and fructose-1,6-bisphosphatase (FBPase) are key gluconeogenic enzymes that participate in the hepatic release of glucose to the circulatory system. It is reported that insulin normally suppresses gluconeogenic enzyme hepatic G6Pase and FBPase [12-14]. Despite hyperinsulinemia, hepatic G6Pase and FBPase activities are higher in db/db mice than in db/+m. Dietary administration of DHEA and that of troglitazone significantly decreased blood glucose and hepatic G6Pase and FBPase activities in db/db mice.

To further elucidate the mechanism of the insulin
sensitizing effect of DHEA at the molecular level, we examined in the present study the effect of DHEA on the expression of genes encoding G6Pase and FBPase.

**Materials and Methods**

**Animals and diet**

Fifteen male C57BL/KsJ-db/db mice and five lean heterozygote littermates db/+m were purchased from Clea Japan, Inc. (Tokyo, Japan). Five mice were placed in one cage and given food and water ad libitum throughout the study. Blood glucose concentration was measured at 1500 h every day with whole blood obtained from tail veins using a portable blood glucose analyzer, Antsense II (Bayer-Sankyo Co., Ltd., Tokyo, Japan). Five-week old db/db mice and db/+m mice were maintained on standard pellet food, Oriental MF obtained from Oriental Co., Ltd. (Tokyo, Japan) for three weeks. The db/db mice were divided into three groups at eight weeks, five for DHEA administration, five for troglitazone administration, and five for control (given standard food). The five db/+m mice were administered standard food. For two weeks either DHEA or troglitazone was administered to mice as 0.4% and 0.2% (W/W) additives, respectively, contained in the standard pellet diet. Each additive was mixed in the standard powder food and pelleted by Oriental Co., Ltd. These doses were the same as in previous studies [2, 14, 15]. Food intake and body weight were determined daily. Livers were removed and weighed at decapitation at ten weeks.

**Reagents**

All reagents and enzymes were of analytical grade and DHEA was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Troglitazone was obtained from Sankyo Co., Ltd. (Tokyo, Japan).

**Enzyme assay and protein determination**

Mice were fasted overnight and then decapitated. The liver from each mouse was frozen immediately in liquid nitrogen and stored at −80°C. Each liver was thawed on ice to be homogenized for enzyme assays. Liver was cut into small pieces with scissors, and homogenized in ice cold homogenizing buffer [50 mM Tris-HCl, pH 7.5, containing 250 mM sucrose and 0.2 mM EDTA] at 10 ml of buffer per 1 g of tissue. The liver was homogenized using a glass/teflon homogenizer. The homogenate was centrifuged at 20,000 g for 20 min. at 4°C, and the 20,000-g supernatant was ultracentrifuged at 105,000 g for one hour at 4°C. The resulting sediments were suspended in 1 ml of homogenizing buffer and used for the assay of G6Pase. The supernatant was used for the assay of FBPase.

All enzyme activities were measured photometrically using a Hitachi U-2000 spectrophotometer (Tokyo, Japan). The activity of FBPase was determined as described by Ulm et al. [16]. The formation of NADPH was followed at 340 nm at 25°C using 6.22 mM−1cm−1 as its extinction coefficient. The G6Pase activity was measured as described by Gierow and Jergil [17]. The formation of quinonemine was followed at 510 nm at 25°C using 6.66 mM−1cm−1 as its extinction coefficient. The enzyme activities were expressed as the nanomoles of substrate converted by one milligram of cytosolic or microsomal protein per minute. Protein was determined using a DC Protein assay kit (Bio-Rad, Hercules, CA, USA). The liver microsomal fraction was solubilized by addition of 0.1% SDS before protein determination.

**Probe preparation**

cDNA was synthesized from total RNA of db/+m mouse liver and amplified by use of the RT-PCR kit (Takara, Tokyo, Japan) and the primers according to the manufacturer’s protocol. The primers for G6Pase (sense, 5′-GCAGCTTCCTGAGGTACCAG-3′, antisense, 5′-TTTGCATGGCGGTTGACTTTA-3′) were selected to amplify sequences corresponding to the nucleotides from +52 to +1199 of the mouse G6Pase [18] (GenBank U00445). The primers for FBPase (sense, 5′-CGTCATGGAGCAGGGCAGGGA-3′, antisense, 5′-TGACGACTGGTGCGTCTCTGTGTT-3′) were selected to amplify sequences corresponding to the nucleotides from +241 to +1125 of the mouse FBPase (GenBank AJ132693). The PCR products were subcloned to pCR2.1 vector (Invitrogen Corporation, Carlsbad, CA, USA). *Escherichia coli* JM109 was transformed with pCR2.1
G6Pase or FBPase. The fragments that were used as a template to make the probe were EcoRI fragments of G6Pase or FBPase subcloned in the pCR2.1 vector. Sequencing confirmed the identity of the appropriately-sized product.

**RNA preparation and Northern blot analysis**

Total RNA was extracted from each liver using Isogen (Nippon Gene, Tokyo, Japan) according to the manufacturer's protocol and 20 μg of total RNA per lane were electrophoresed and transferred to a nylon membrane (Hybond N+, Amersham, Buckinghamshire, U.K.) by capillary transfer. The corresponding mRNA was detected by hybridization with a 32P cDNA probe. The hybridization signals were analyzed with a Bio-image analyzer (BAS 2000; Fuji, Tokyo).

**Other analytical methods**

Blood for determining insulin levels was obtained from vessels exposed at decapitation. Plasma insulin was measured using an ELISA insulin kit provided with the mouse insulin as a standard, purchased from Seikagaku Corporation (Tokyo, Japan).

**Statistical analysis**

Data are expressed as mean±SE. Differences in mean values between the lean db/+m and control db/db mice were statistically analyzed by Student’s t test. Differences in mean values between the db/db mice control group and other groups were statistically analyzed using the Tukey-Kramer test. Differences in P values of less than 0.05 were considered significant.

**Results**

**Blood glucose, plasma insulin, body and liver weight, and food intake**

As shown in Table 1, the blood glucose level of the control db/db mice was higher than that of db/+m mice. Administration of DHEA improved hyperglycemia of db/db mice, supporting the results of previous reports [2, 3]. Troglitazone also significantly lowered blood glucose in db/db mice. Plasma insulin concentration in diabetic db/db mice was about 14 times as high as that of non-diabetic db/+m mice. Plasma insulin concentration in DHEA and troglitazone-treated db/db mice tended

| Table 1. Plasma Insulin, Body and Liver Weight, and BG (Blood Glucose) in Four Groups of Mice |
|----------------------------------|-----------------|-----------------|------------------|-----------------|
|                                  | db/+m           | db/db           | db/db + DHEA     | db/db + TGZ     |
| Insulin (pg/ml)                  | 479±8           | 6982±1116*      | 5212±616         | 5326±359        |
| Initial body weight (g)          | 22.3±0.2        | 35.9±0.7*       | 35.9±0.6         | 35.7±1.1        |
| Final body weight (g)            | 24.1±0.3        | 39.0±0.6*       | 39.3±0.6         | 44.5±1.4*       |
| Liver weight (g)                 | 1.50±0.43       | 2.04±0.17*      | 2.58±0.93*       | 2.65±0.34*      |
| BG (mg/dl)                       | 157±5           | 538±46*         | 365±43*          | 310±55*         |

Each value is the mean±SE. *P<0.05 vs. db/+m mice and #P<0.05 vs. db/db mice. db/+m; db/+m mice treated with standard food (n=5), db/db; db/db mice treated with standard food (n=5), db/db+DHEA; db/db mice treated with DHEA (n=5), and db/db+TGZ; db/db mice treated with troglitazone (n=5).

| Table 2. Comparison of Hepatic G6Pase and FBPase Activities Among Four Groups of Mice |
|----------------------------------|-----------------|-----------------|------------------|-----------------|
|                                  | db/+m           | db/db           | db/db + DHEA     | db/db + TGZ     |
| G6Pase (nmol/mg protein/min)     | 69±8            | 172± 5*         | 128±7*           | 105±12*         |
| FBPase (nmol/mg protein/min)     | 108±4           | 222±12*         | 135±4*           | 128±3*          |

TGZ represents troglitazone. Each column and bar represents mean±SE. *P<0.05 vs. control db/+m mice and #P<0.05 vs. control db/db mice.
to be lower, although it was not statistically significant. There was no significant difference in the initial body weight among the three randomly divided groups of db/db mice. After treatment, only troglitazone increased the body weight of db/db mice by 14% in comparison to the control db/db mice. Liver weight was heavier in db/db mice compared to the db/+ m mice. DHEA increased the liver weight in db/db mice by 26%. Troglitazone also increased the liver weight in db/db mice by 30%.

There was no significant difference in food intake between the db/db mice that consumed DHEA or troglitazone (data not shown).

**Enzyme activities**

Hepatic G6Pase and FBPase activities of the db/db mice were elevated by 149% and 106%, respectively, compared to the db/+ m mice (Table 2).

Administration of DHEA to the db/db mice induced a significant decrease in hepatic G6Pase and FBPase activities by 26% and 39%, respectively. Troglitazone also decreased these enzyme activities by 39% and 42%, respectively.

**Northern blot analysis**

Figure 1 shows the results of Northern blot analysis of hepatic G6Pase and FBPase mRNA. The level of hepatic G6Pase mRNA in db/db mice was significantly higher than that in db/+ m mice and administration of DHEA or troglitazone decreased the G6Pase mRNA close to the level of the db/+ m mice. In contrast, the level of hepatic FBPase mRNA in db/db mice was not statistically different from that in db/+ m mice, and administration of DHEA or troglitazone caused no change in the FBPase mRNA level.

**Discussion**

Since there has been no report that investigates the hypoglycemic effect of DHEA in db/db mice since Coleman et al. reported this effect in db/db mice [2, 3], we previously reported that the activities of G6Pase and FBPase are increased despite the increased plasma levels of insulin in db/db mice, although insulin inhibits the action of this gluconeogenic enzyme in the liver of normal mice [11]. Administration of DHEA or troglitazone to db/db mice
suppressed the elevated enzyme activity. As shown in Table 1, the activities of G6Pase and FBPase in db/db mice are much higher than that in non-diabetic db/+ m mice. The results were in accordance with our previous report [11]. Administration of DHEA or troglitazone improved hyperglycemia and decreased the enzyme activity. Davies et al. [19] reported that the G6Pase mRNA level in isolated hepatocytes from BB/Zucker obese-diabetic rats was elevated compared to non-diabetic rats and that troglitazone reduced the expression of G6Pase mRNA. However, there have been no reports that evaluate the effects of DHEA on the expression of G6Pase and FBPase in db/db mice. Therefore, we evaluated the mRNA level of these two hepatic gluconeogenic enzymes in db/db mice to further elucidate the effect of DHEA as an insulin sensitizing agent.

It is reported that insulin has a repressive effect on G6Pase mRNA [12-14]. Despite hyperinsulinemia, the hepatic G6Pase mRNA level in db/db mice was elevated as shown Table 1 and Figure 1. Administration of DHEA and that of troglitazone significantly decreased the expression of this enzyme in db/db mice. These changes in the G6Pase mRNA level are in accordance with the changes in enzyme activity. In contrast, the hepatic FBPase mRNA level of db/db mice was not significantly higher than that of db/+ m mice even though the enzyme activity was significantly higher in db/db as shown in Table 2 and Figure 1. These results indicate that the mechanism of the elevation and suppression of the two hepatic gluconeogenic enzymes is different in these animals. They suggest that the G6Pase gene is at least one target for the hypoglycemic effect of DHEA as an insulin sensitizer in db/db mice, and that the elevation of G6Pase mRNA may be a possible cause of insulin resistance.

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glucose-6-phosphatase protein levels are increased in streptozotocin-induced diabetes. 


