Dehydroepiandrosterone Suppresses the Elevated Hepatic Glucose-6-Phosphatase and Fructose-1,6-Bisphosphatase Activities in C57BL/Ksj-*db/db* Mice

Comparison With Troglitazone

Kazutaka Aoki, Tatsuya Saito, Shinobu Satoh, Koji Mukasa, Mizuki Kaneshiro, Satsuki Kawasaki, Atsushi Okamura, and Hisahiko Sekihara

The effect of dehydroepiandrosterone (DHEA) on the hepatic and muscle glucose metabolizing enzymes and on blood glucose were investigated in insulin-resistant diabetic C57BL/KsJ-*db/db* mice and their heterozygote littermates (*db*/+m). The results were compared with those after troglitazone administration under the same conditions. Despite hyperinsulinemia, hepatic glucose-6-phosphatase (G6Pase) and fructose-1,6-bisphosphatase (FBPase) activities are higher in *db/db* than in *db*/+m mice. Dietary administration of DHEA and that of troglitazone for 15 days to respective groups of five mice each significantly decreased blood glucose in *db/db* mice and hepatic G6Pase and FBPase activities in both *db/db* and *db*/+m mice. Hepatic G6Pase and FBPase activities showed a linear relationship with blood glucose in all groups of mice, suggesting that the activities of G6Pase and FBPase are closely related to blood glucose levels. Because androstenedione, a DHEA metabolite, barely affected either of these enzyme activities or blood glucose in *db/db* mice, the actions of DHEA, which are similar to those of troglitazone, are presumed to be caused by DHEA itself. DHEA is considered to be a modulating agent for the activities of hepatic gluconeogenic enzymes in *db/db* mice. *Diabetes* 48:1579–1585, 1999

C57BL/KsJ-*db/db* mice have been shown to become obese, hyperglycemic, and hyperinsulinemic (1). Coleman et al. (2,3) reported that dietary administration of dehydroepiandrosterone (DHEA), a major adrenal androgen, to *db/db* mice induced remission of hyperglycemia and increased insulin sensitivity. Cleary and Mohan (4–8) showed the effects of DHEA on mitochondrial respiration in rats. McIntosh and Berdanier (9,10) proposed that the effects of DHEA are mediated by futile substrate cycling in hepatocytes of BHE/cdb rats. Many studies suggest that DHEA increases the sensitivity of insulin (11–13). These studies state that changes in hepatic glucose metabolism are a possible factor in the hypoglycemic effect of DHEA, but changes in the hepatic or muscle enzyme activities in *db/db* mice have not been studied. The present study further evaluates the problem by systematically investigating the changes induced by administration of DHEA in key enzyme activities of hepatic glycolysis and gluconeogenesis as well as the corresponding enzyme activities in the muscle. Because troglitazone is a hypoglycemic agent that has been recently introduced into clinical medicine to treat diabetic patients who are insulin resistant, we also evaluated the activities of the same enzymes in the liver and muscle after the administration of troglitazone.

RESEARCH DESIGN AND METHODS

Animals and diets. Male C57BL/KsJ-*db/db* mice and their lean heterozygote littermates (*db*/+m) were purchased from Clea Japan (Tokyo). Four or five mice were placed in one cage and given food and water ad libitum throughout the study. Blood glucose concentrations were measured at 3:00 P.M. every day with whole blood obtained from tail veins using a portable blood glucose analyzer (Antisense II; Bayer-Sankyo, Tokyo). A total of 20 *db/db* mice and 14 *db*/+m mice 13 weeks of age were maintained on standard pellet food (Oriental MF; Oriental, Tokyo) for 2 weeks. The *db/db* mice were divided into four groups of five mice each for DHEA administration, troglitazone administration, insulin administration (given standard food), and control (given standard food). The *db*/+m mice were divided into three groups for DHEA administration (n = 5), troglitazone administration (n = 4), and control (given standard food) (n = 5). DHEA or troglitazone was administered to mice for 15 days as 0.4 and 0.2% (wt/wt) additives, respectively, contained in the standard pellet diet. Each additive was mixed in the standard powder food and pelleted by Oriental. These doses were the same as in previous studies (2,14). Insulin suspension containing 100 U/ml human isophane insulin (Novo Nordisc Pharma, Tokyo) was administered subcutaneously at 3:00 P.M. for 15 days, depending on the blood glucose: 15 U for <200 mg/dl, 20 U for 201–300 mg/dl, 25 U for 301–400 mg/dl, 30 U for 401–500 mg/dl, and 35 U for >501 mg/dl.

In another experiment, 14 *db/db* mice 12 weeks of age were maintained on the same standard pellet food described above for 2 weeks. The *db/db* mice were divided into two groups of seven mice each for androstenedione administration and control (given standard food). Androstenedione was administered for 15 days to mice as a 0.4% (wt/wt) additive, contained in the standard pellet diet. Food intake and body weight were determined daily. Epididymal adipose tissue, liver, and soleus muscle were removed and weighed at decapitation.

From the Third Department of Internal Medicine, Yokohama City University School of Medicine, Yokohama, Japan.

Address correspondence and reprint requests to Kazutaka Aoki, Third Department of Internal Medicine, Yokohama City University School of Medicine, 3-9 Fuku-ura, Kanazawa-ku, Yokohama 236-0004, Japan. E-mail: caa90130@pop06.odn.ne.jp.

Received for publication 30 October 1998 and accepted in revised form 28 April 1999.

DHEA, dehydroepiandrosterone; FBPase, fructose-1,6-bisphosphatase; G6Pase, glucose-6-phosphatase; GK, glucokinase; HK, hexokinase; PFK, phosphofructokinase; PK, pyruvate kinase.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/48/8/1579/338004/10426376.pdf by guest on 13 May 2025

TABLE 1
Plasma insulin, DHEA, and blood glucose in six groups of mice on day 15

	db/+m			*db/db*		
	Standard food	Treated with DHEA	Treated with troglitazone	Standard food	Treated with DHEA	Treated with troglitazone
n	5	5	4	5	5	5
Insulin (pg/ml)	979 ± 51	910 ± 72	1,120 ± 210	4,634 ± 309*	11,449 ± 2,005†	3,234 ± 260
DHEA (ng/ml)	1.22 ± 0.22	10.68 ± 2.65*	1.46 ± 0.59	1.74 ± 0.17	19.94 ± 8.97†	1.96 ± 0.30
Blood glucose (mg/dl)	159 ± 2	145 ± 2	131 ± 4*	557 ± 5*	349 ± 9†	324 ± 9†

Data are means ± SE. *P < 0.05 vs. *db*/+m mice; †P < 0.05 vs. *db/db* mice.

Reagents. All reagents and enzymes were of analytical grade and purchased from Sigma Chemical (St. Louis, MO), or Wako Pure Chemical Industries (Tokyo). Troglitazone was obtained from Sankyo (Tokyo).

Enzyme assay and protein determination. Mice were fasted overnight and then decapitated. Freshly excised muscles were homogenized. The liver from each mouse was frozen immediately in liquid nitrogen and stored at –80°C. After 1 week, about half of each liver was thawed on ice to be homogenized for enzyme assays other than glucose-6-phosphatase (G6Pase). Two weeks after homogenizing the first half, the remaining liver was thawed to prepare microsomal suspension for the G6Pase assay. The muscle and liver were cut into small pieces with scissors and homogenized in ice-cold buffer (0.1 mol/l Tris-HCl, pH 7.5, containing 0.15 mol/l KCl, 5 mmol/l EDTA, 5 mmol/l dithiothreitol, and 5 mmol/l $MgSO_4$) (10 ml buffer per 1 g tissue). In the microsomal preparation for the G6Pase assay, 50 mmol/l Tris-HCl, pH 7.5, containing 250 mmol/l sucrose and 0.2 mmol/l EDTA, was used as the homogenizing buffer (10 ml buffer per 1 g tissue). The liver and muscle were separately homogenized using a glass/Teflon homogenizer or a polytron homogenizer. Except for the assay of liver G6Pase, the homogenate was centrifuged at 105,000*g* for an hour at 4°C, and the supernatant was collected. The 105,000*g* supernatant was divided into six portions, one of which was supplemented with 30 mmol/l potassium fluoride and used for the assay of phosphofructokinase (PFK) activity on the same day. The rest was stored at –80°C. For the assay of G6Pase, the liver microsomal fraction was prepared as follows: the homogenate obtained as above 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 an hour at 4°C. The resulting sediments were suspended in 1 ml homogenizing buffer and used for the assay of G6Pase.

All enzyme activities were measured photometrically using a Hitachi U-2000 spectrophotometer (Tokyo). Except for the assay of G6Pase, the formation or disappearance of either NADH or NADPH was measured by following the absorbance at 340 nm at 25°C. The extinction coefficient used for the pyridine nucleotide reduced form was 6.22 $mmol \cdot l^{-1} \cdot cm^{-1}$. The activities of hexokinase (HK) and glucokinase (GK) were determined as described by Pilkis (15). The activity of PFK was determined by the method reported by Kemp (16). The activity of pyruvate kinase (PK) was determined as described by Blair et al. (17). The activity of fructose-1,6-bisphosphatase (FBPase) was determined as described by Ulm et al. (18). The PEPCK activity was measured using the method described by Colombo et al. (19). The activity of G6Pase was measured as described by Gierow and Jergil (20). The formation of quinoneimine was followed at 510 nm and 25°C using 6.66 $mmol \cdot l^{-1} \cdot cm^{-1}$ as its extinction coefficient. The enzyme activities were expressed as the number of substrate molecules converted by 1 mg cytosolic or microsomal protein per minute. Protein was determined using a DC Protein assay kit (Bio-Rad, Hercules, CA). The liver microsomal fraction was solubilized by addition of 0.1% SDS before protein determination.

Other analytical methods. Blood for determining insulin and DHEA levels was obtained from vessels exposed at decapitation. Plasma insulin was measured using an enzyme-linked immunosorbent assay insulin kit provided with the mouse insulin as a standard, purchased from Seikagaku (Tokyo). Plasma DHEA was quantified by direct radioimmunoassay using a highly specific double-antibody method from Diagnostic Systems Laboratories (Webster, TX). The percent cross-reactivity of DHEA antiserum expressed as the ratio of DHEA concentration is as follows: DHEA = 100%; DHEA-sulfate = 0.02%; progesterone = 0.045%; corticosterone, estriol, estrone, and estradiol = not detectable.

Statistical analysis. Data from DHEA and troglitazone treatments were analyzed by two-way analysis of variance followed by Duncan's multiple range test. Data from insulin- or androstenedione-treated mice were analyzed by Student's t test. Differences with P values of <0.05 were considered significant. Regression analysis was performed using data on the mean blood glucose level of the last 5 days and hepatic G6Pase or FBPase activities.

RESULTS

Respective effect of DHEA, troglitazone, and insulin administration on blood glucose, plasma DHEA, plasma insulin, and food intake. As shown in Table 1, DHEA improved hyperglycemia of *db/db* mice, supporting the results of previous reports (2,3). Troglitazone as well as insulin significantly lowered blood glucose in *db/db* mice. In *db*/+m mice, in which the mean blood glucose value was lower than that of the *db/db* mice by 400 mg/dl, only troglitazone significantly lowered the blood glucose level. The blood glucose level of DHEA-treated *db*/+m mice tended to be lower than that of controls, but the difference was not statistically significant (Table 1). There was no difference in plasma DHEA concentration between the *db/db* and *db*/+m mice. The plasma DHEA concentration was clearly increased in the DHEA-treated *db/db* mice compared with two other groups of *db/db* mice in Table 1. Plasma DHEA levels were

TABLE 2
Body composition in six groups of mice on day 15

	db/+m			*db/db*		
	Standard food	Treated with DHEA	Treated with troglitazone	Standard food	Treated with DHEA	Treated with troglitazone
n	5	5	4	5	5	5
Liver weight (g)	0.99 ± 0.22	1.14 ± 0.03	1.04 ± 0.05	2.01 ± 0.03*	2.43 ± 0.12†	2.65 ± 0.10†
Muscle weight (mg)	152 ± 9	141 ± 5	161 ± 6	114 ± 7*	110 ± 8	93 ± 2
Epididymal fat weight (mg)	174 ± 41	122 ± 6	155 ± 18	972 ± 48*	977 ± 17	1,006 ± 26
Initial body weight (g)	27.8 ± 1.0	27.1 ± 0.4	28.3 ± 1.7	48.1 ± 1.1*	49.2 ± 0.8	49.4 ± 1.2
Final body weight (g)	29.3 ± 1.8	29.0 ± 0.4	30.6 ± 3.6	55.7 ± 0.2*	55.1 ± 0.8	59.8 ± 1.5†

Data are means ± SE. *P < 0.05 vs. *db*/+m mice; †P < 0.05 vs. *db/db* mice.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/48/8/1579/338004/10426376.pdf by guest on 13 May 2025

also significantly elevated in the DHEA-treated *db*/+m mice compared with the two other groups of *db*/+m mice.

The plasma insulin concentration in diabetic *db/db* mice was about 4.7 times higher than that of nondiabetic *db*/+m mice. The plasma insulin concentration in DHEA-treated *db/db* mice was increased by about 2.5 times over that of the control group of *db/db* mice, while plasma insulin concentrations in troglitazone-treated *db/db* mice tended to be lower, although the difference was not statistically significant. There were no significant changes in the plasma insulin concentration among the three groups of *db*/+m mice (Table 1). There was no significant difference in food intake between the *db/db* mice that consumed DHEA or troglitazone and their respective controls, but the *db/db* mice treated with insulin consumed 75% of that consumed by the controls (data not shown). There was no significant difference in food intake in the three groups of *db*/+m mice.

Effect of DHEA and troglitazone on body composition. As shown in Table 2, no significant difference in initial body weight, soleus muscle, and epididymal adipose tissue weight was found in the three groups of *db*/+m mice and the three groups of *db/db* mice. The liver and epididymal adipose tissue weights of *db/db* mice increased compared with those of *db*/+m mice, and the soleus muscle weight of *db*+m mice was heavier than that of *db/db* mice. After treatment, only troglitazone increased the body weight of *db/db* mice by 7% in comparison to the control *db/db* mice. DHEA increased the liver weight only in *db/db* mice by 20%. Troglitazone also increased the liver weight only in *db/db* mice by 30%.

Comparison of enzyme activities between the *db/db* and the *db*/+m mice. The activities of hepatic PK, G6Pase, and FBPase of the *db/db* mice were elevated by 166, 135, and 98%, respectively, compared with the *db*/+m mice, whereas hepatic PEPCK activity was decreased by 35% (Figs. 1 and 2). The muscle enzymes PFK and FBPase were increased by 360 and 210%, respectively (Figs. 3 and 4).

Respective effect of DHEA, troglitazone, and insulin on enzyme activities in *db/db* mice. Administration of DHEA to the *db/db* mice induced significant decreases in hepatic G6Pase and FBPase activities by 25 and 29%, respectively.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/48/8/1579/338004/10426376.pdf by guest on 13 May 2025

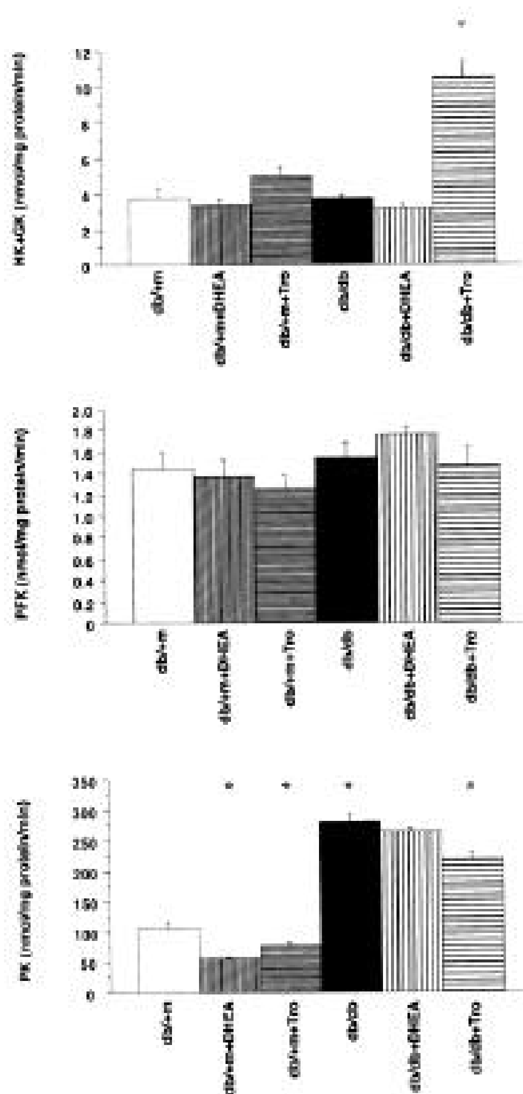

FIG. 1. Comparison of hepatic glycolytic enzyme activities among six groups of mice. Tro, troglitazone. Each column and bar represents mean ± SE. $*P < 0.05$ vs. control *db*/+m mice; $\#P < 0.05$ vs. control *db/db* mice.

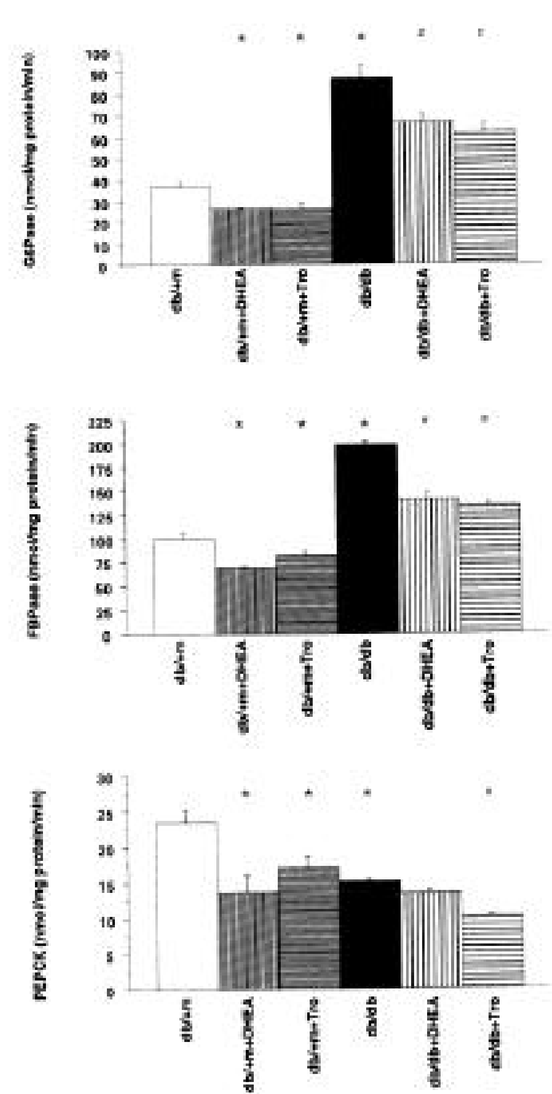

FIG. 2. Comparison of hepatic gluconeogenic enzyme activities among six groups of mice. Tro, troglitazone. Each column and bar represents mean ± SE. $*P < 0.05$ vs. control *db*/+m mice; $\#P < 0.05$ vs. control *db/db* mice.

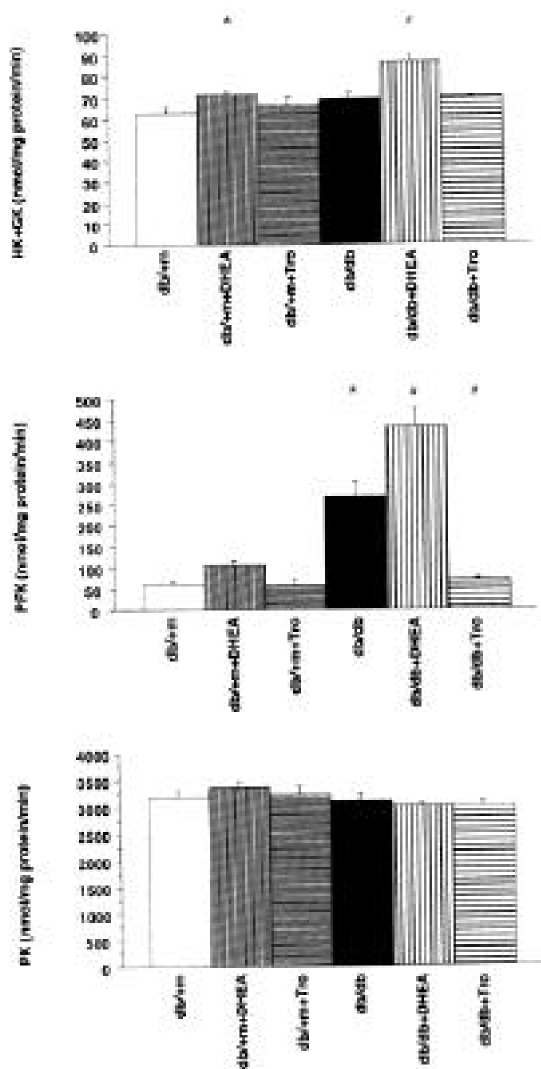

FIG. 3. Comparison of muscle glycolytic enzyme activities among six groups of mice. Tro, troglitazone. Each column and bar represents mean ± SE. *P < 0.05 vs. control *db*/+m mice; #P < 0.05 vs. control *db/db* mice.

Troglitazone or insulin also decreased these two enzyme activities. Troglitazone decreased hepatic PEPCK and PK activity and increased hepatic HK and GK activity by 181% (Figs. 1 and 2; Table 3). With regard to muscle enzymes, DHEA increased PFK activity by 62% and insulin also increased PFK activity, whereas troglitazone decreased it. DHEA increased muscle HK and GK activity by 26% (Fig. 3; Table 3).

Effect of DHEA and troglitazone on enzyme activities in the *db*/+m mice. In the *db*/+m mice, DHEA significantly decreased hepatic G6Pase, FBPase, PEPCK, and PK activities

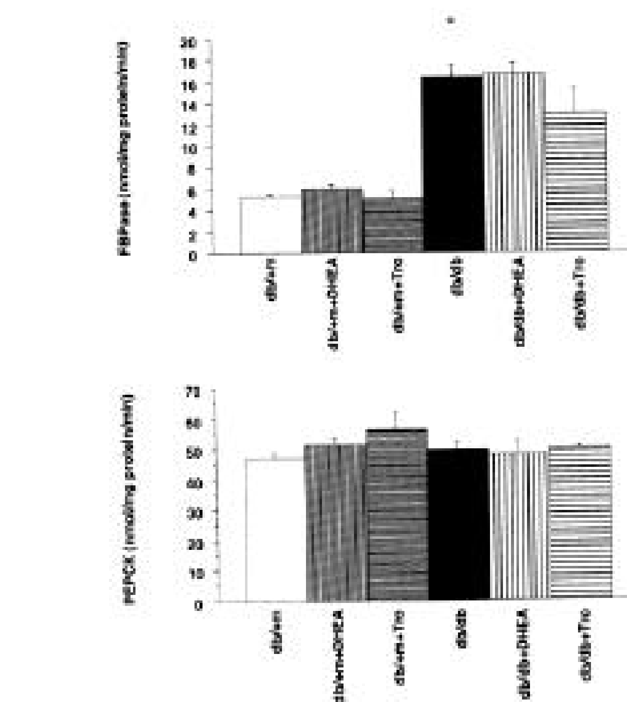

FIG. 4. Comparison of muscle gluconeogenic enzyme activities among six groups of mice. Tro, troglitazone. Each column and bar represents mean ± SE. *P < 0.05 vs. control *db*/+m mice; #P < 0.05 vs. control *db/db* mice.

by 30, 32, 42, and 49%, respectively. Troglitazone also decreased these four hepatic enzyme activities (Figs. 1 and 2). DHEA increased muscle HK and GK activity by 14% (Fig. 3).

Relationship between hepatic gluconeogenic enzyme activities (G6Pase and FBPase) and blood glucose. The linear relationship between activities of hepatic gluconeogenic enzymes, G6Pase, and FBPase, and the mean blood glucose values for the last 5 days of treatment are shown in Fig. 5. A good correlation was obtained between the activity of G6Pase or FBPase and blood glucose levels.

Effects of androstenedione on blood glucose and enzyme activities in *db/db* mice. Androstenedione lowered blood glucose of *db/db* mice by only 5% (Table 4). Muscle PFK activity was increased by 410%, and hepatic FBPase activity was decreased by 11%. The activity of hepatic G6Pase was not changed. There was no difference in food intake between the androstenedione-treated and control *db/db* mice.

DISCUSSION

As there have been no reports that investigate the hypoglycemic effect of DHEA in *db/db* mice since Coleman et al. reported this effect in *db/db* mice (2), we administered DHEA to *db/db* mice

TABLE 3
The effect of insulin on blood glucose and three enzyme activities on day 15

	n	Blood glucose (mg/dl)	Hepatic G6Pase (nmol · mg^{-1} protein · min^{-1})	Hepatic FBPase (nmol · mg^{-1} protein · min^{-1})	Muscle PFK (nmol · mg^{-1} protein · min^{-1})
db/db					
Standard food	5	557 ± 5	87 ± 6	198 ± 3	267 ± 37
Treated with insulin	5	348 ± 12*	48 ± 3*	154 ± 4*	708 ± 139*

Data are means ± SE. *P < 0.05 vs. control *db/db* mice.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/48/8/1579/338004/10426376.pdf by guest on 13 May 2025

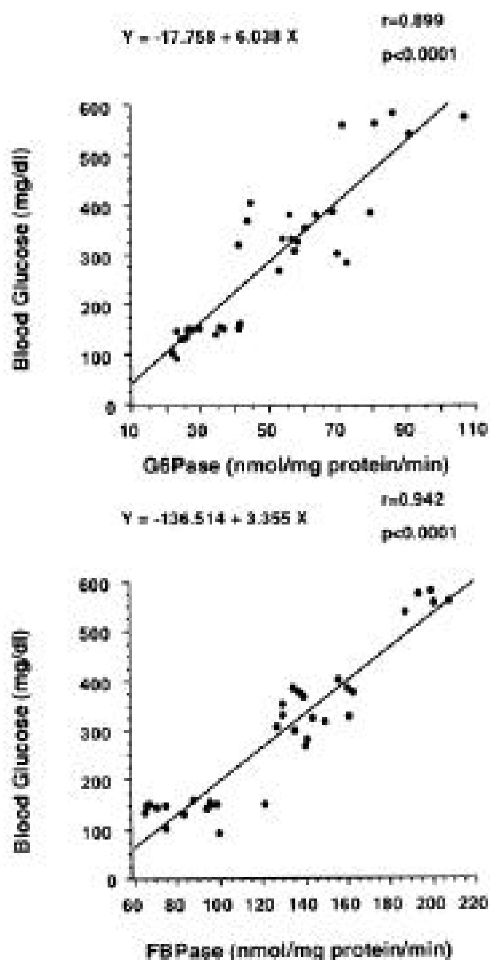

FIG. 5. Regression analysis between hepatic G6Pase or FBPase activities and mean blood glucose for the final 5 days. Regression lines were drawn using data from all mice except for those on androstenedione treatment in this study.

and evaluated the changes in blood glucose level to confirm the findings of Coleman et al. The improvement of hyperglycemia was obtained (Table 1). The degree of the hypoglycemic effect induced by 400 mg/kg DHEA was almost the same as that caused by 200 mg/kg troglitazone, which is now in general clinical use. Therefore, in the present study, we systematically measured the glycolytic enzyme activities (HK and GK, PFK, and PK) and gluconeogenic enzyme activities (G6Pase, FBPase, and PEPCK) of liver and muscle in *db/db* and *db*/+m mice as the first step in elucidating the mechanism of the hypoglycemic effect of DHEA in *db/db* mice that Coleman et al. discovered in 1982. We also administered troglitazone and measured the activities of the same enzymes to compare the effect of DHEA with that of troglitazone.

At first, we compared hepatic enzyme activities in *db/db* mice with those in *db*/+m mice. In *db/db* mice, as expected, the hepatic PK activity was increased and hepatic PEPCK activity was decreased compared with *db*/+m mice. These changes are consistent with hyperinsulinemia. Despite hyperinsulinemia, hepatic G6Pase and FBPase activities in *db/db* mice, which were normally suppressed by the action of insulin (21), were increased compared with *db*/+m mice. The increased activities of G6Pase and FBPase are considered to be important for the development of hyperglycemia in *db/db* mice, as suggested in previous reports (22–25).

As to the respective effects of DHEA and troglitazone on the gluconeogenic and glycolytic enzyme activities, it is reported that DHEA decreased hepatic HK, GK, PK, and FBPase activities and increased hepatic G6Pase activities in normal Sprague-Dawley rats (26). McIntosh et al. (10) reported that DHEA decreased hepatic PEPCK activity in diabetic BHE/cdb rats. It is also reported that troglitazone decreased hepatic FBPase activity and did not change PFK activity in *db/db* mice (14). As no extensive studies exist regarding enzymatic changes caused by DHEA or troglitazone administration in diabetic *db/db* mice, we systematically investigated the respective changes in enzyme activities induced by DHEA and troglitazone. DHEA decreased the elevated hepatic G6Pase and FBPase activities in *db/db* mice. Troglitazone also decreased the elevated hepatic G6Pase and FBPase activities in *db/db* mice. The effects of DHEA and troglitazone on these hepatic enzymes were similar to that of insulin, because the decrease in these two enzyme activities (Fig. 2) was also observed in insulin-treated *db/db* mice (Table 3).

There was a significant positive correlation between hepatic G6Pase or FBPase activity and blood glucose level in our study, as shown in Fig. 5. This correlation suggests that the reduction in the elevated hepatic G6Pase and FBPase activities induced by the respective administration of DHEA, troglitazone, and insulin obtained in the present study may lead to decreased hepatic glucose production in *db/db* mice. The previous reports indicating that DHEA or troglitazone reduces hepatic glucose production (10,14,27–31) support the inference. In addition to the reduction in G6Pase and FBPase activities, troglitazone increased hepatic HK and GK activity in *db/db* mice. This increase in enzyme activities may promote hepatic glycolysis in *db/db* mice. The decrease in hepatic HK and GK activity and increase in hepatic G6Pase activity induced by DHEA administration found in normal rats (26) is different from our results obtained in diabetic mice. Probably the action of DHEA is different between normal rats and *db/db* or *db*/+m mice. The reduction in hepatic G6Pase

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/48/8/1579/338004/10426376.pdf by guest on 13 May 2025

TABLE 4
The effect of androstenedione on blood glucose and three enzyme activities on day 15

	n	Blood glucose (mg/dl)	Hepatic G6Pase (nmol · mg^{-1} protein · min^{-1})	Hepatic FBPase (nmol · mg^{-1} protein · min^{-1})	Muscle PFK (nmol · mg^{-1} protein · min^{-1})
db/db					
Standard food	7	578 ± 7	85 ± 5	221 ± 8	190 ± 18
Treated with androstenedione	7	548 ± 5*	97 ± 3	196 ± 9*	969 ± 48*

Data are means ± SE. *$P < 0.05$ vs. control *db/db* mice.

and FBPase activities induced by DHEA and troglitazone was also found in *db*/+m mice in which the blood glucose was lower than in *db/db* mice. The decrease induced by each of these two agents was comparable to that in *db/db* mice when expressed as percentage of each control. The findings suggest that the changes induced by DHEA and troglitazone in the *db/db* mice were not secondary to the reduced glucose toxicity caused by DHEA or troglitazone.

There is no report on the muscle enzyme activity in *db/db* mice. Therefore, we evaluated the muscle glycolytic and gluconeogenic enzyme activities in *db/db* mice and the effect of DHEA and troglitazone on these enzyme activities. The muscle PFK activity was increased in *db/db* mice compared with *db*/+m mice. The increase was in accordance with hyperinsulinemia. In *db/db* mice, DHEA further increased muscle PFK activity (Fig. 3), and androstenedione increased the activity (Table 4). In contrast, troglitazone decreased the activity (Fig. 3). The activity of muscle FBPase in *db/db* mice was increased compared with that in *db*/+m mice. DHEA or troglitazone did not change muscle FBPase activity in *db*/+m and *db/db* mice (Fig. 4), although they suppressed the activity of the same enzyme in the liver. There was no significant correlation between muscle enzyme activities and blood glucose levels.

It is commonly recognized that troglitazone promotes the glucose disposal rate and reduces hepatic glucose production in type 2 diabetic patients (30,31), but recently it was reported that the effects of troglitazone were exerted on muscles and did not change the glucose production in the liver in type 2 diabetic patients (32). According to the results obtained in the present study using *db/db* mice, the effects of troglitazone are considered to be exerted mainly on the liver, judging from the changes of hepatic G6Pase and FBPase activities.

To determine whether DHEA itself or a metabolite is effective in the improvement of hyperglycemia, we administered androstenedione, which is considered to be converted from DHEA in vivo. Androstenedione lowered blood glucose only to a slight degree (Table 4), suggesting that the hypoglycemic effect is due to DHEA itself.

Increased plasma insulin by DHEA in *db/db* mice was considered to be due to prevention of islet cell atrophy, as previously demonstrated histologically by Coleman et al. (2). However, as most of the enzyme activity changes induced by DHEA in *db/db* mice are reproduced in *db*/+m, in which plasma insulin did not change, it is reasonable to infer that the effect of DHEA on enzymes is not through an increasing insulin concentration. DHEA is known to improve the effectiveness of insulin (2,3,11–13); thus even if the concentration of insulin in the heterozygous mice does not increase on treatment with DHEA, the normal concentration of insulin could be responsible for the decreased blood sugar.

In humans, DHEA is synthesized through the action of 17 α-hydroxylase in the adrenals, but this enzyme is not expressed in the mouse adrenal, resulting in meager DHEA production in mice (33). Plasma DHEA in DHEA-treated mice is considered to be derived from the pellet diet that contained the DHEA and did not far exceed the upper limit of physiologic range in humans (about 7 ng/ml). It is reported that hyperinsulinemic diabetic patients have lower serum DHEA levels compared with nonhyperinsulinemic diabetic patients or normal subjects, suggesting that DHEA may have something to do with the regulation of blood glucose levels in humans (34). The findings obtained in this study may provide further evidence for the participation of DHEA in the regulation of blood glucose. The details of the mechanisms of DHEA in the regulation of blood glucose are to be evaluated in future studies.

REFERENCES

1. Gunnarsson R: Function of the pancreatic B-cell during the development of hyperglycemia in mice homozygous for the mutations “diabetes” (db) and “misty” (m). *Diabetologia* 11:431–438, 1975
2. Coleman DL, Leiter EH, Schwizer RW: Therapeutic effects of dehydroepiandrosterone (DHEA) in diabetic mice. *Diabetes* 31:830–833, 1982
3. Coleman DL, Schwizer RW, Leiter EH: Effect of genetic background on the therapeutic effects of dehydroepiandrosterone (DHEA) in diabetes-obesity mutants and aged normal mice. *Diabetes* 33:26–32, 1984
4. Mohan PF, Cleary MP: Effect of short term DHEA administration on liver metabolism of lean and obese rats. *Am J Physiol* 255:E1–E8, 1988
5. Mohan PF, Cleary MP: Dehydroepiandrosterone and related steroids inhibit mitochondrial respiration in vitro. *Int J Biochem* 21:1103–1107, 1989
6. Cleary MP: Effect of dehydroepiandrosterone treatment on liver metabolism in rats. *Int J Biochem* 22:205–210, 1990
7. Mohan PF, Cleary MP: Short-term effects of dehydroepiandrosterone treatment in rats on mitochondrial respiration. *J Nutr* 121:240–250, 1991
8. Cleary MP: The antiobesity effect of dehydroepiandrosterone in rats. *Proc Soc Exp Biol Med* 196:8–16, 1991
9. McIntosh MK, Berdanier CD: Antiobesity effects of dehydroepiandrosterone are mediated by futile substrate cycling in hepatocytes of BHE/cbd rats. *J Nutr* 121:2037–2043, 1991
10. Berdanier CD, McIntosh MK: Further studies on the effects dehydroepiandrosterone of hepatic metabolism in BHE rats. *Proc Soc Exp Biol Med* 192:242–247, 1989
11. Ladriere L, Laghmich A, Malaisse-Lagae F, Malaisse WJ: Effect of dehydroepiandrosterone in hereditarily diabetic rats. *Cell Biochem Funct* 15:287–292, 1997
12. Kimura M, Tanaka S, Yamada Y, Kikuchi Y, Yamakawa T, Sekihara H: Dehydroepiandrosterone decreases serum TNF α and restores insulin sensitivity: independent effect from secondary weight reduction in genetically obese Zucker fatty rats. *Endocrinology* 139:3249–3253, 1998
13. Mukasa K, Kaneshiro M, Aoki K, Okamura J, Saito T, Satoh S, Sekihara H: Dehydroepiandrosterone (DHEA) ameliorates the insulin sensitivity in older rats. *J Steroid Biochem Mol Biol* 67:355–358, 1998
14. Fujiwara T, Okuno A, Yoshioka S, Horikoshi H: Suppression of hepatic gluconeogenesis in long-term troglitazone treated diabetic KK and C57BL/KsJ-db/db mice. *Metabolism* 44:486–490, 1995
15. Pilkis SJ: Glucokinase of rat liver. *Methods Enzymol* 42:31–39, 1975
16. Kemp RG: Phosphofructokinase from rabbit liver. *Methods Enzymol* 42: 67–71, 1975
17. Blair JB, Cimbala MA, Foster JL, Morgan RA: Hepatic pyruvate kinase. *J Biol Chem* 251:3756–3762, 1976
18. Ulm EH, Pogell BM, De Maine MM, Libby CB, Benkovic SJ: Fructose-1,6-diphosphatase from rabbit liver. *Methods Enzymol* 42:369–374, 1975
19. Colombo G, Carlson GM, Lardy HA: Phosphoenolpyruvate carboxykinase (guanosine triphosphate) from rat liver cytosol: separation of homogeneous forms of the enzyme with high and low activity by chromatography on agarose-hexane-guanosine triphosphate. *Biochemistry* 17:5321–5329, 1978
20. Gierow P, Jergil B: Spectrophotometric method for glucose-6-phosphate phosphatase. *Methods Enzymol* 89:44–47, 1982
21. Pilkis SJ, Granner DK: Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. *Annu Rev Physiol* 54:885–909, 1992
22. Chang AY, Schneider DI: Abnormalities in hepatic enzyme activities during development of diabetes in db mice. *Diabetologia* 6:274–278, 1970
23. Chan TM, Young KM, Hutson NJ, Brumley FT, Exton JH: Hepatic metabolism of genetically diabetic (db/db) mice. I. Carbohydrate metabolism. *Am J Physiol* 229:1702–1711, 1975
24. Kodama H, Fujita M, Yamazaki M, Yamaguchi I: The possible role of age-related increase in the plasma glucagon/insulin ratio in the enhanced hepatic gluconeogenesis and hyperglycemia in genetically diabetic (C57BL/KsJ-db/db) mice. *Jpn J Pharmacol* 66:281–287, 1994
25. Hotta K, Kawajima M, Ono A, Nakajima H, Shingu R, Miyagawa J, Namba M, Hanafusa T, Noguchi T, Kono N, Matsuzawa Y: Disordered expression of hepatic glycolytic and gluconeogenic enzymes in Otsuka Long-Evans Tokushima fatty rats with spontaneous long-term hyperglycemia. *Biochim Biophys Acta* 1289:145–149, 1996
26. Mayer D, Weber E, Moore MA, Letsch I, Filsinger E, Bannasch P: Dehydro-

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/48/8/1579/338004/10426376.pdf by guest on 13 May 2025

epiandrosterone induced alternations in rat liver carbohydrate metabolism. *Carcinogenesis* 9:2039–2043, 1988
27. Lee MK, Miles PD, Khoursheed M, Gao KM, Moossa AR, Olefsky JM: Metabolic effects of troglitazone on fructose-induced insulin resistance in the rat. *Diabetes* 43:1435–1439, 1994
28. Fulgencio JP, Kohl C, Girard J, Pegorier JP: Troglitazone inhibits fatty acid oxidation and esterification, and gluconeogenesis in isolated hepatocytes from starved rats. *Diabetes* 45:1556–1562, 1996
29. Raman P, Foster SE, Stokes MC, Strenge JK, Judd RL: Effect of troglitazone (Rezulin) on fructose 2,6-bisphosphate concentration and glucose metabolism in isolated rat hepatocytes. *Life Sci* 62:89–94, 1998
30. Suter SL, Nolan JJ, Wallace P, Gumbiner B, Olefsky JM: Metabolic effects of new oral hypoglycemic agent CS-045 in NIDDM subjects. *Diabetes Care* 15: 193–203, 1992
31. Maggs DG, Buchanan TA, Burant CF, Cline G, Gumbiner B, Hsueh WA, Inzucchi S, Kelley D, Nolan J, Olefsky JM, Polonsky KS, Silver D, Valiquett TR, Shulman GI: Metabolic effects of troglitazone monotherapy in type 2 diabetes mellitus: a randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 128:176–185, 1998
32. Inzucchi SE, Maggs DG, Spollett GR, Page SL, Rife FS, Walton V, Shulman GI: Efficacy and metabolic effects of metformin and troglitazone in type II diabetes mellitus. *N Engl J Med* 338:867–872, 1998
33. van Weerden WM, Bierings HG, van Steenbrugge GJ, de Jong FH, Scroder FH: Adrenal gland of mouse and rat do not synthesize androgens. *Life Sci* 50: 857–861, 1992
34. Yamaguchi Y, Tanaka S, Yamakawa T, Kimura M, Ukawa K, Yamada Y, Ishihara M, Sekihara H: Reduced serum dehydroepiandrosterone levels in diabetic patients with hyperinsulinemia. *Clin Endocrinol* 49:377–383, 1998

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/48/8/1579/338004/10426376.pdf by guest on 13 May 2025