

# Effect of Genetic Background on the Therapeutic Effects of Dehydroepiandrosterone (DHEA) in Diabetes-Obesity Mutants and in Aged Normal Mice

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## SUMMARY

Dehydroepiandrosterone (DHEA) was fed at 0.1–0.4% in the diet to genetically diabetic (*db/db*) or obese (*ob/ob*) C57BL/KsJ (BL/Ks) or C57BL/6J (BL/6) mice. Treatment of BL/Ks-*db/db* or *ob/ob* mice with 0.4% DHEA prevented hyperglycemia, islet atrophy, and severe diabetes associated with this inbred background, but did not affect weight gain and food consumption. Homozygous obese (*ob*) or diabetes (*db*) mice on the BL/6 background were more sensitive to DHEA, and the mild, transient hyperglycemia associated with *ob* or *db* gene expression on the BL/6 inbred background could be prevented by 0.1% DHEA. Both body weight and food consumption were decreased in BL/6 mutants maintained on 0.1% DHEA whereas this effect was not seen in BL/Ks mutants fed up to 0.4% DHEA. Early therapy with 0.4% DHEA, initiated at 2 wk of age, prevented the development of most diabetes symptoms and decreased the rate of weight gain in pups of all genotypes. In addition to therapeutic effects on both obese mutants, DHEA effected significant changes in an aging study using normal BL/6 female mice. Four weeks of DHEA treatment initiated at 2 yr of age improved glucose tolerance and at the same time reduced plasma insulin to a “younger” level. This suggests that DHEA may act in insulin-resistant mutant mice and in aging normal mice to increase the sensitivity to insulin. *DIABETES* 33:26–32, January 1984.

**D**ehydroepiandrosterone (androst-5-en-3 $\beta$ -ol-17-one, DHEA) and its sulfate derivative are the major adrenal secretory products in humans, yet their biologic functions remain unclear. Decreased secretion of DHEA has been reported to occur with advancing age<sup>1</sup> and in women with certain types of breast cancer.<sup>2</sup> Chronic treatment with DHEA in the diet has been shown to

increase longevity in rodents by retarding the development of specific diseases associated with the particular strain or mutant. Specifically, obesity is ameliorated or prevented in the *A<sup>y</sup>* mutant mouse<sup>3</sup> and in the Zucker rat;<sup>4</sup> tumor susceptibility is decreased in the *A<sup>y</sup>* mutant mouse maintained on the mammary-tumor-susceptible C3H strain,<sup>5</sup> and the development of the autoimmune disease in NZB mice associated with aged mice of this strain is delayed.<sup>6</sup> Continued treatment of rodents with up to 1% DHEA in the diet throughout their entire lifespan had no detrimental effects and the beneficial effects were all reversible when treatment was withdrawn.<sup>3,7</sup> It has long been known that caloric restriction of an otherwise adequate diet has an ameliorative effect on most age-associated renal, cardiac, vascular, and neoplastic lesions<sup>8,9</sup> and can significantly extend lifespan.<sup>10</sup> Chronic feeding of DHEA appears to have all of these same effects while having no reported effects on food intake. Recent data<sup>7</sup> suggest that DHEA may act in adult rats by increasing energy expenditure rather than reducing food intake.

DHEA has been shown to be an inhibitor of the enzyme, glucose-6-phosphate dehydrogenase (G6PDH), the rate-limiting enzyme of the hexose monophosphate shunt<sup>11</sup> and the enzyme that provides the major source of extramitochondrial reduced NADP required for maintenance of lipogenesis. This interference with the supply of reduced NADP could explain the effect of DHEA on lipogenesis, while a decreased supply of the product of this reaction (pentose phosphate) could be associated with the antiproliferative properties and the inhibition of tumor development. On the other hand, any nonproductive energy expenditure produced by DHEA would divert calories away from specific metabolic pathways and mimic caloric restriction without actually decreasing food intake.

Two mutations, obese (*ob*) and diabetes (*db*), produce similar diabetic-obesity states in mice, the severity of which depends on unknown background factors inherent in the inbred strains in which the mutations are maintained.<sup>12–14</sup> On the C57BL/KsJ (BL/Ks) background the syndrome is characterized by obesity and a severe life-shortening diabetes, whereas on the C57BL/6J (BL/6) background both muta-

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tions produce severe obesity with a transient well-compensated diabetes. Feeding BL/Ks mutants restricted amounts of a complete diet (50% of normal food intake) has little effect on obesity or diabetes development.<sup>15</sup>

Feeding DHEA (0.4%) in the diet rapidly restored the hyperglycemia in BL/Ks diabetes mice to normal, and improved glucose tolerance without affecting the degree of obesity or food consumption.<sup>16</sup> Therapeutic effects were seen even when treatment was instituted in the near terminal stages of the diabetes condition. The most striking effect of DHEA was almost total prevention of the severe atrophy and degeneration of the islet of Langerhans typical of the diabetes syndrome in BL/Ks mutants. DHEA treatment converted this severe diabetes to a well-compensated diabetes with severe obesity and insulin resistance, a syndrome very similar to that seen when either the obese or diabetes mutation is maintained on the BL/6 inbred background. This report compares the effects of DHEA in the two different diabetes-obesity syndromes associated with either the obese (*ob*) or diabetes (*db*) mutations maintained on the BL/6 and BL/Ks backgrounds.

## MATERIALS AND METHODS

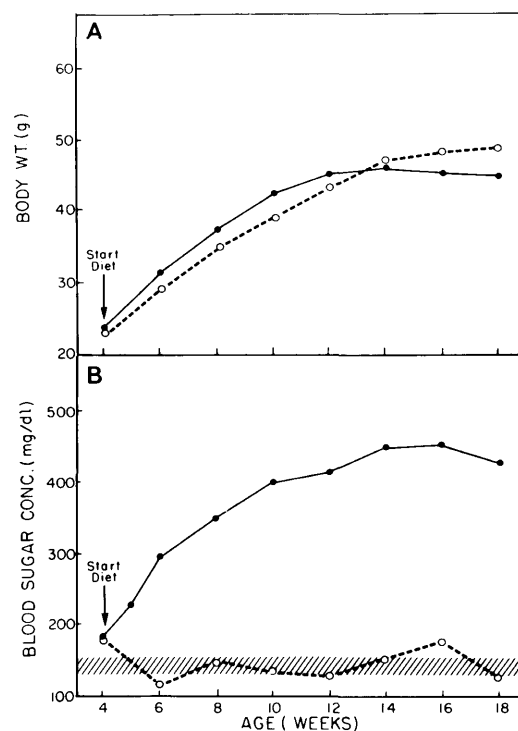
**Animals.** Diabetes (*db/db*) and normal (+/+) control mice of the BL/6 strain and obese (*ob/ob*) mice of the BL/Ks strain were produced in our research colony. Obese (*ob/ob*) mice of the BL/6 strain were obtained from the Animal Resources Division of the Jackson Laboratory. Male mice were used in most studies except where otherwise indicated in the text. To test the efficacy of DHEA on preweaning mutant mice before development of any symptoms of diabetes or obesity, BL/Ks or BL/6 misty diabetes mice (*m db/m db*), which have the coat color misty (*m*) and diabetes (*db*) genes coupled on the same chromosome, were used for ease of identification of future diabetes mice (grey pelage). The development of these congenic strains has been described.<sup>17</sup> The introduction of the misty (*m*) gene does not affect the development of the diabetes syndrome in either strain. In these studies, heterozygous breeding pairs were fed chow containing 0.4% DHEA to provide maximum exposure of the developing fetuses to DHEA and to permit access by the pups to only a DHEA-containing diet at the time of spontaneous weaning. The aged mice, two groups of 12 BL/6 female mice aged 1 and 2 yr, respectively, were obtained from the research colony of Dr. David E. Harrison (The Jackson Laboratory).

In each study, weanling, preweanling, or aged mice were divided into groups, one fed chow alone (Old Guilford 96) and others fed powdered chow into which DHEA had been incorporated. The powdered diets were fed in food cups that were filled every second day. In food consumption studies, the powdered chow containing DHEA was repelleted in a hydraulic press (5000 pounds/sq. in.) and weighed amounts of these pellets were fed each day. Daily food consumption was determined by weighing the amount left after 24 h.

**Analytical procedures.** Mice were weighed weekly at the time of bleeding for determination of the blood sugar concentration. Plasma immunoreactive insulin concentrations were determined periodically during the treatment period and at the time of termination of each experiment. After the mice were killed, the pancreas was removed, weighed, and

one-half was fixed in Bouin's solution for subsequent histologic study and morphologic analysis, while the other half was homogenized in acid-ethanol (1.5% concentrated HCl in 70% ethanol) to determine pancreatic insulin content. Blood glucose concentration, insulin concentration, and glucose tolerance tests were carried out as previously described.<sup>18</sup> The islet area and percent of granulated beta-cells were measured using an Optomax IV image analyzer system (Optomax, Hollis, New Hampshire). Hydrated 5- $\mu$ m sections of Bouin's fixed tissues were stained with aldehydefuchsin to detect beta-cells. Islets for measurement were selected at random until data from 8–15 individual islets were accumulated per mouse. These were combined to give an average value for islet area and percent of granulated beta-cells. Data are expressed as mean  $\pm$  SEM for the individual means of mice from various treatment groups. Statistical comparisons were calculated using Student's *t* test. Differences were considered significant at  $P < 0.01$ .

G6PDH activity was measured in red blood cell lysates and high-speed supernatants from liver, kidney, and adipose tissue according to a standard procedure.<sup>11</sup> The reaction was started with aliquots of supernatant or lysate (usually 0.1 ml) and run at room temperature with the reduced NAPD being measured at 340 nm on a Beckman DBG T recording spectrophotometer (Beckman Instruments, Fullerton, California). Steroids were added in dioxane. 16- $\alpha$ -Bromoepiandrosterone was prepared by refluxing epiandrosterone (Sigma, St. Louis, Missouri) with cupric bromide in methanol for 24 h.<sup>20,21</sup>



**FIGURE 1. (A)** Effect of DHEA treatment on weight gain in BL/Ks diabetes mice. Data are based on eight male mice. Chow fed (●—●) and 0.4% DHEA (○—○). **(B)** Effect of DHEA treatment on blood sugar concentrations in BL/Ks diabetes mice. Chow fed (●—●) and 0.4% DHEA (○—○). Cross-hatched region represents normal range.

TABLE 1  
Comparison of the effects of DHEA feeding on diabetic and normal BL/6 and BL/Ks mice\*

Strain Genotype (N)	DHEA (%)	Food consumption (g)	B.S. (mg/dl)	Plasma IRI ( $\mu$ U/ml)	Pancreatic insulin (U/g)	Granulated beta-cells (%)
<b>BL/Ks</b>						
+ / + (6)	0.0	4.04 $\pm$ 0.20	131 $\pm$ 2.04	42.4 $\pm$ 1.96	3.84 $\pm$ 0.63	68.8 $\pm$ 7.10
+ / + (3)	0.4	3.95 $\pm$ 0.18	101 $\pm$ 1.42	51.4 $\pm$ 3.34	3.89 $\pm$ 0.13	79.4 $\pm$ 5.80
<i>db/db</i> (11)	0.0	4.19 $\pm$ 0.16	>450	205 $\pm$ 28.9	1.06 $\pm$ 0.25	4.06 $\pm$ 1.08
<i>db/db</i> (11)	0.4	6.21 $\pm$ 0.11†	156 $\pm$ 18.9	2374 $\pm$ 1883.0†	10.40 $\pm$ 0.08†	44.2 $\pm$ 6.18†
<i>db/db</i> (8)	0.2	ND	194 $\pm$ 22.9	730 $\pm$ 215.0†	3.76 $\pm$ 0.85	28.0 $\pm$ 14.40†
<b>BL/6</b>						
+ / + (6)	0.0	3.82 $\pm$ 0.15	140 $\pm$ 4.00	37.3 $\pm$ 5.42	2.67 $\pm$ 0.17	76.3 $\pm$ 2.35
+ / + (5)	0.4	3.03 $\pm$ 0.13	118 $\pm$ 3.51	37.4 $\pm$ 8.92	2.29 $\pm$ 0.12	70.3 $\pm$ 3.42
<i>db/db</i> (16)	0.0	4.82 $\pm$ 0.17	140 $\pm$ 15.00	2302 $\pm$ 453.70	8.50 $\pm$ 1.89	14.4 $\pm$ 4.37
<i>db/db</i> (10)	0.4	2.78 $\pm$ 0.18	111 $\pm$ 9.23	1414 $\pm$ 344.60	8.97 $\pm$ 1.48	49.9 $\pm$ 6.75†
<i>db/db</i> (6)	0.1	ND	126 $\pm$ 12.70	>4000†	9.48 $\pm$ 1.84†	26.4 $\pm$ 2.84†

\*Data represent average values  $\pm$  SEM for groups of male mice treated from weaning with the respective dietary treatment, at which time the mice were killed for determinations of the physiologic and morphometric parameters.

†Significantly different from normal controls or untreated diabetes (*db/db*) mutants ( $P < 0.01$ ).

**RESULTS**

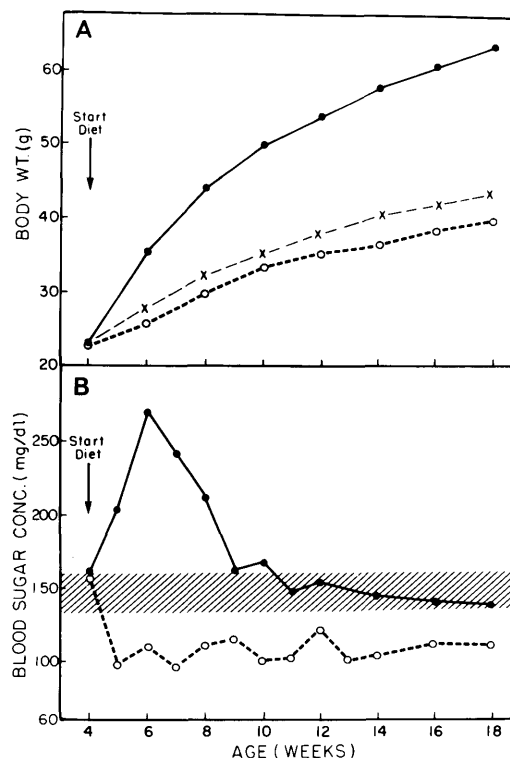
**Effects of postweaning diet treatment.** Diabetes (*db*) mutants maintained in the BL/Ks inbred strain background and fed 0.4% DHEA gained weight at a rate not significantly different from those mutants fed chow (Figure 1A). The food consumption did not change in BL/Ks mutants fed 0.4% DHEA. Changes in average blood sugar concentrations of mutants fed chow alone and chow containing 0.4% DHEA are shown in Figure 1B, while changes in other physiologic parameters are seen in Table 1. DHEA treatment (0.4%) prevented the development of severe diabetes; blood sugar remained within the normal range during the entire treatment period. DHEA (0.2%) was somewhat less effective than 0.4% (Table 1) with respect to prevention of hyperglycemia. The average blood sugar concentration in 4 mutants treated with 0.2% DHEA for 12 wk was 194  $\pm$  22.9 mg/dl, compared with 156  $\pm$  18.9 mg/dl for 11 mutants maintained on 0.4% DHEA (Table 1). DHEA treatment of normal BL/Ks mice for 16 wk after weaning decreased the average blood sugar concentration, but had little effect on plasma insulin concentration, pancreatic insulin content, or the percent granulated beta-cells (Table 1).

One group of 8 older BL/Ks diabetes mutants with established, but not terminal, hyperglycemia (blood sugar 340  $\pm$  10.5 mg/dl) was treated with 0.4% DHEA for 4 wk. After only 2 wk of treatment, blood sugar concentrations had normalized in all mice. The average blood sugar concentration at the time of death (4 wk) was 155  $\pm$  11.2 mg/dl. Plasma insulin concentrations at death were 1857  $\pm$  472  $\mu$ U/ml in treated mutants compared with 205  $\pm$  28.9  $\mu$ U/ml in similarly aged untreated mutants. Pancreatic insulin content had increased to within the normal range and the percent granulated beta-cells (30.5  $\pm$  4.9%) was markedly increased, although not to normal values. Islet atrophy was not obvious in the treated mutants. The degree of islet intactness and extent of beta-cell granulation (31.3  $\pm$  9.6%) approached that seen in mice treated with DHEA from weaning (44.2  $\pm$  6.2%).

The therapeutic effects of 0.4% DHEA were also studied in a group of 6 male obese (*ob*) mutants on the BL/Ks inbred background (data not shown). The effect in treated BL/Ks-

*ob/ob* mutants was similar if not identical in all parameters to those reported here, and in a previous publication,<sup>16</sup> for BL/Ks-*db/db* mutants; no change in the rate of weight gain or decrease in food consumption was observed, whereas blood sugar concentrations were restored to normal as long as treatment continued.

In contrast to BL/Ks mutants, BL/6 mutants maintained on chow containing 0.4% DHEA gained much less weight



**FIGURE 2. (A)** Effect of DHEA treatment on weight gain in BL/6 diabetes male mice. Points are significantly different ( $P < 0.01$ ) for all groups after 4 wk of age. Data based on eight mice per group. Chow fed (●—●), 0.1% DHEA (X—X), and 0.4% DHEA (O—O). **(B)** Effect of DHEA treatment on blood sugar concentration in BL/6 diabetes mice. Chow fed (●—●) and 0.4% DHEA (O—O). Cross-hatched region represents normal range.

than mutants fed chow alone. Figure 2A shows the average growth curve for groups of 8 male BL/6-*db/db* mice fed either chow alone or chow containing 0.1% and 0.4% DHEA. The final body weight attained in the chow-fed mutants was nearly 60 g compared with only 35 g in those mutants fed 0.4% DHEA. Treatment with 0.1% DHEA produced an intermediate rate of weight gain to that seen for chow-fed mutants and those fed 0.4% DHEA (Figure 2A). The weights of all mice after 4 wk of treatment with either concentration of DHEA were all significantly decreased ( $P < 0.01$ ) compared with those mutants fed chow alone. The weights of BL/6 diabetes mutants fed 0.1% DHEA were significantly ( $P < 0.01$ ) increased over that of mice fed 0.4% DHEA.

Studies carried out with a group of 6 BL/6 *ob/ob* mutants gave similar results (data not shown) except that the final body weight of obese mutants exceeded 70 g and that of mutants treated with 0.4% DHEA was 48 g. Although no determination was made of actual body composition, it was obvious at autopsy that most of the weight decrease in all BL/6 mutants fed DHEA could be attributed to a marked decrease in the accretion of adipose tissue.

The effects of DHEA treatment on blood sugar concentration in BL/6 *db/db* mutants is shown in Figure 2B and Table 1. Mutants treated before the transient rise in blood sugar that typically occurs between 4 and 8 wk of age failed to exhibit hyperglycemia at any time after initiation of treatment. Instead, blood sugar decreased to values below normal for the duration of the study. The blood sugar profile of treated and untreated BL/6 (*ob/ob*) mutants (data not shown) was identical to that seen in BL/6 *db/db* mice. Those mutants fed only 0.1% DHEA responded equally as well as those fed 0.4% with respect to the changes in blood sugar concentrations. When treatment was initiated at 6–8 wk in BL/6 diabetes or obese mutants (after mild hyperglycemia had occurred), normal glycemia was restored within a week and remained normal as long as treatment continued.

Groups of normal mice of either strain fed 0.4% DHEA from weaning always gained slightly less weight compared with mice fed chow alone. After a 12-wk treatment period, 10 normal BL/6 male mice fed 0.4% DHEA gained less (1.4 g) compared with mice fed chow (2.6 g). A similar reduction in weight gain was seen in BL/Ks normal mice fed DHEA. These differences were not significant. All organs appeared normal on gross examination at autopsy, except the adipose tissue, which was extremely small. Both mutant and normal BL/Ks mice fed DHEA had normal food intakes, while BL/6 mice decreased food intake in response to DHEA treatment

(Table 1). No attempt was made in this study to pair feed DHEA-treated mutants that amount of food eaten by BL/6 mutants. Reduction of daily food intake from 6.21 to 2.78 g/day would have undoubtedly led to a significant reduction in the rate of weight gain to more nearly approximate the decreased rate of weight gain seen in treated BL/6 mutants. Feeding of 0.4% DHEA had a greater anorectic effect on the reduction of food intake in the hyperphagic BL/6 mutants (42% reduction) than in normal mice (21% reduction).

Plasma insulin concentrations of diabetes mutants maintained on the BL/6 background typically increased from about 3 times normal (150  $\mu\text{U/ml}$ ) to over 2000  $\mu\text{U/ml}$  as the disease progressed and severe insulin resistance developed (Table 1). BL/6 diabetes mice fed 0.4% DHEA did not show as great a rise in plasma insulin concentration as those fed chow (1414 versus 2302  $\mu\text{U/ml}$ ) (Table 1). In contrast, BL/6 mutants fed only 0.1% DHEA maintained the largest and most consistent increase in plasma insulin concentration ( $>4000 \mu\text{U/ml}$ ).

Histologic examination of the pancreas revealed large hyperactive islets with enlarged sinusoids in all BL/6 *db/db* mice regardless of diet. Pancreatic insulin content (Table 1) in chow-fed BL/6 mutants was much higher than that seen in either chow- or DHEA-treated normal (+/+) BL/6 mice even though the beta-cells of mutants were markedly degranulated when compared with normal mice. Feeding 0.4% DHEA to BL/6 mutants increased the extent of beta-cell granulation but was without effect on pancreatic insulin content (Table 1). Feeding of DHEA at 0.1% produced an intermediate effect with respect to beta-cell granulation (Table 1) and rate of weight gain (Figure 2B), whereas it was fully as effective as 0.4% with respect to blood sugar changes (Table 1). The inhibitory effect of DHEA on weight gain and food consumption in BL/6 mutants is in sharp contrast to the effect in BL/Ks mutants reported above, where DHEA normalized the blood sugar, but was without effect on either rate of weight gain or food consumption.

**Effect of DHEA exposure during the peri- and postnatal period.** Our initial preclinical studies involved feeding chow containing 0.4% DHEA to known heterozygous breeding pairs of each strain to assure maximal exposure of developing litters to any effects of DHEA. No pregnancies occurred when these pairs were maintained on chow-containing DHEA. Litters were not obtained even when diets containing DHEA were fed to pregnant females as late as midterm in pregnancy (12 days). Therefore, nursing dams were switched to the chow containing 0.4% DHEA 10–14

TABLE 2  
Effects of feeding DHEA during the peri- and postnatal period\*

Genotype (no.)	Plasma IRI ( $\mu\text{U/ml}$ )	Pancreatic insulin (U/g)	Granulated beta-cells (%)	Mean islet area ( $\mu\text{m}^2$ )
BL/6 +/? (6)	84.3 $\pm$ 14.2	4.74 $\pm$ 0.59	63.3 $\pm$ 4.42	8282 $\pm$ 1098
BL/6 <i>mdb/mdb</i> (6)	405 $\pm$ 107.3†	6.79 $\pm$ 1.69	62.4 $\pm$ 5.18	8804 $\pm$ 540
BL/Ks +/? (9)	81 $\pm$ 9.63	3.74 $\pm$ 0.38	75.2 $\pm$ 1.35	10809 $\pm$ 1103
BL/Ks <i>mdb/mdb</i> (6)	477 $\pm$ 122.5†	16.6 $\pm$ 4.92†	53.4 $\pm$ 6.13†	24683 $\pm$ 6123†

\*Data represent mean  $\pm$  SEM for groups of mice of both sexes fed DHEA (0.4%) at 10–14 days of age. Mice were killed at 2 mo of age after 6 wk of treatment when the various physiologic and morphologic parameters were measured.

†Significantly different from normal ( $P < 0.01$ ).

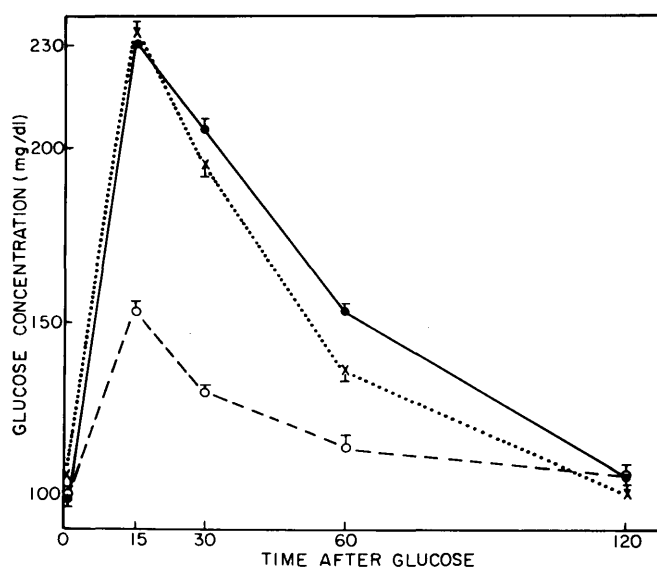
days after birth of the litter. All pups in litters from female mice fed DHEA grew more slowly than those fed chow alone. After weaning at 4 wk, the presence of the *db* gene carried in linkage with the *m* gene in misty (*m/m*) weanlings was verified by their increased body weights irrespective of the diet fed. Blood sugar concentrations in all mutants, either BL/6 or BL/Ks, remained normal as long as DHEA treatment continued. Insulin concentration, in both plasma and pancreas of mutants, was elevated (Table 2) compared with those obtained from normal littermates. Mean islet area was the same in treated BL/6 mutants, whereas it was increased in BL/Ks mutants. Percent granulated beta-cells was equal in mice of the BL/6 strain and decreased in mutants of the BL/Ks strain. The extent of granulation in these younger normal mice of both strains was not as great as that typical of older normal mice (approximately 60–70%). This was in sharp contrast to the severely reduced percentage seen in either BL/Ks or BL/6 *db/db* mice fed chow alone (Table 1). Islet atrophy typical of chow-fed BL/Ks mutants never occurred in DHEA-treated pups of either strain and correlated with the complete absence of the physiologic symptoms of diabetes.

**Aging studies.** There were no significant differences in fed and fasted (16 h) blood sugar concentrations between the 1- and the 2-yr-old female BL/6 mice at the start of treatment and both groups responded normally to a glucose challenge. After 4 wk on the DHEA-containing diet, a distinct improvement in glucose handling was observed in the 2-yr-old group fed DHEA (Figure 3). No differences in response to glucose were seen for the younger groups, fed either chow or DHEA, or the old group fed chow alone. Plasma immunoreactive insulin concentrations at death (after 8 wk on diet) were normal in all groups (80–110  $\mu$ U/ml) except for the chow-fed 2-yr-old mice, which had an average plasma insulin con-

centration of  $263 \pm 3.6$   $\mu$ U/ml. In spite of this markedly elevated plasma insulin seen in the old untreated group, the average fed blood sugar concentration was no different from that of any of the other groups (80–100 mg/dl). The plasma insulin responses, 30 min after glucose challenge, were equivalent in both DHEA-treated ( $53.1 \pm 5.9$   $\mu$ U/ml) and untreated ( $51.0 \pm 6.6$   $\mu$ U/ml) young mice, whereas insulin secretion in response to glucose challenge was enhanced in both treated ( $105.3 \pm 12.6$   $\mu$ U/ml) and untreated ( $122 \pm 22.2$   $\mu$ U/ml) older mice.

**Route of administration of DHEA.** In one set of experiments using BL/Ks *db/db* mutants and controls, DHEA was incorporated into the diet in 2% of agar to obtain cubes of food, which would be more convenient for studies on food consumption. The expected anti-hyperglycemic effects of DHEA treatment were not obvious after a 1-mo trial period with the agarized, DHEA-containing diet. When these mice were switched to a powdered diet containing DHEA, without agar, hyperglycemia was checked and blood sugar was held at around 250 mg/dl (data not shown). Thus, the initial gene-induced metabolic damage that occurred during the presentation of DHEA in agarized form was not completely repaired by subsequent feeding of the steroid in powdered diets.

Treatment of BL/Ks diabetes mutants with DHEA by injection subcutaneously in propylene glycol (12.5 mg in 0.25 ml/day), or a lower dose in peanut oil (50  $\mu$ g in 50  $\mu$ l, twice a week) for 2 wk, was also without effect. Bromoepiandrosterone (16  $\alpha$ ), an analogue of DHEA, is 60 times more active than DHEA with respect to the inhibition of G6PDH.<sup>20</sup> No effect of this compound on blood sugar or rate of weight gain was seen when injected in amounts up to 10 mg/day or when fed at 0.4% in the diet of BL/Ks mice. Both of these treatments caused up to a 50% inhibition of G6PDH activity in extracts of liver, kidney, adipose tissue, and reticulocytes. In contrast, feeding of DHEA at 0.4% concentration had no effect on G6PDH activity in similar tissue extracts but had a dramatic effect on both the control of diabetes in BL/Ks mutants and the regulation of weight gain in BL/6 mutants.



**FIGURE 3.** Glucose tolerance tests in 2-yr-old BL/6 chow-fed (●—●), 1-yr-old fed either chow or 0.4% DHEA (X··X), and 2-yr-old fed 0.4% DHEA (○---○). Points represent average values obtained from six female mice of each age and dietary treatment. The 15- and 30-min values from the DHEA-treated mice were significantly less ( $P < 0.01$ ) than those same points for all other groups.

## DISCUSSION

This study has confirmed and extended our previous finding<sup>16</sup> that DHEA is a potent anti-hyperglycemic and anti-diabetic agent when fed to mice with inherited obesity-glucose intolerance syndromes. Furthermore, an improved glucose tolerance was observed in very old normal mice (>2 yr) treated with DHEA for 4 wk compared with untreated aged mice. While improvements in glucose tolerance of *db/db* or *ob/ob* mice as a result of DHEA feeding were not dependent on inbred strain background, the anti-obesity and anorectic effects of this steroid (when fed after weaning) have been limited to the BL/6 inbred background. The BL/Ks inbred strain, although closely related to BL/6 nevertheless differs at multiple genetic loci; presumably these differences account for the resistance of the strain to the weight- and appetite-suppressant effects observed in mutants on the BL/6 inbred background. The background differences probably reflect subtle metabolic differences, since the DHEA diet introduced at the earliest preweaning stages did effect reductions in rate of BL/Ks mutant weight gain comparable to that seen in BL/6 mutants.

Normal mice showed no apparent adverse effects to DHEA treatment. Food consumption was not decreased in normal BL/Ks mice whereas normal BL/6 mice decreased food intake somewhat. Normal mice of both strains treated with DHEA gained weight at a slightly slower rate than normal and had distinctly smaller adipose stores that probably represent decreased adipocyte size.<sup>22</sup> Increased oxygen consumption and normal food intake coupled with decreased weight gain has been reported in normal rats fed 0.6% DHEA,<sup>10</sup> suggesting that DHEA-treated animals may have developed a mechanism whereby calories are utilized non-productively. The increased rate of weight gain and high food intake in mutants of the BL/Ks strain and decreased food intake, coupled with a decreased rate of weight gain in mutants of the BL/6 strain in our studies, do not suggest any DHEA-mediated change in metabolic efficiency. Studies by others have shown beneficial effects of DHEA without any changes in food intake.<sup>3,5,10</sup> Our results suggest that the response with regard to food consumption observed depends on some interaction of the DHEA treatment with the inbred background of the mouse.

An attractive hypothesis to explain the anti-obesity effects of DHEA would be via inhibition of G6PDH, with the consequential lack of reduced NADP required for increased rates of lipid biosynthesis. However, our studies showed that DHEA fed at 0.4%, while effective as an anti-hyperglycemic and an anti-obesity agent, failed to produce tissue concentrations sufficient to inhibit G6PDH. In contrast, a more potent G6PDH inhibitor (16 $\alpha$ -bromoepiandrosterone), either fed or injected, was capable of inhibiting tissue G6PDH activity and yet had no effect on the development of either diabetes or obesity. Although it is difficult to draw conclusions from enzyme assays conducted in vitro with extracts removed from the DHEA-containing milieu of the living organism, it would seem that the inhibition of G6PDH by DHEA can be dissociated from its anti-diabetogenic effect. DHEA is a precursor of both androgen and estrogen. Therefore, some of the differential effects of DHEA between the BL/6 and BL/Ks inbred backgrounds could be related to differences in the metabolism of sex steroids. Indeed, we have reported previously that, in certain inbred strains, sex is a major determinant of diabetes syndrome severity.<sup>13</sup> That the endogenous balance of sex steroids underlies this was suggested by our findings in CBA/Lt-*db/db* mice. On this genetic background, *db/db* males develop a lethal "BL/Ks-type" of diabetes, while female *db/db* mice are diabetes resistant and develop a "BL/6-type" of obesity syndrome.<sup>14</sup> Injection of ovarian sex steroids (progesterone and 17  $\beta$ -estradiol) completely blocked diabetes induction in males (hyperglycemia, islet atrophy), but not increases in body weight or food consumption. Estrogens are known to synergize the hypoglycemic role of insulin, suppress the glycogenolytic action of epinephrine,<sup>23</sup> reduce adipose tissue mass and lipoprotein lipase activity,<sup>24</sup> and influence appetite.<sup>25</sup> Thus, DHEA may be acting through estrogen to express its effects on glucose tolerance in mutant mice of both sexes, and in the old normal female mice in our aging study. Preliminary studies (unpublished data) suggest that DHEA, either fed or injected, can be converted to both testosterone and estrogen. These data argue against sex hormones, per se, as being solely responsible for the beneficial effect of DHEA and suggest that

the action of DHEA may be related to the effects of sex steroids acting synergistically with other, as yet unknown, DHEA metabolites.

The improvement in glucose tolerance in female mice older than 2 yr of age treated with DHEA, but not in younger mice, is of interest. The 2-yr-old chow-fed mice had increased basal concentrations of insulin coupled with normal blood sugar concentration, whereas on glucose stimulus the DHEA-treated mice but not the chow-fed mutants showed a significantly improved glucose tolerance with the same increase in plasma insulin concentration. These studies suggest that DHEA or its metabolites may be acting by decreasing any insulin resistance associated with advanced age, or by increasing glucose metabolism or clearance by some other mechanism in these old mice. Alternatively, DHEA in the diet may be having a direct effect on intestinal absorption of nutrients, especially carbohydrates, in both normal and mutant mice. We have previously found that beta-cell pathogenesis in BL/Ks *db/db* mice was entrained by the presence of dietary carbohydrate and that carbohydrate-free diets were palliative.<sup>26</sup> Thus, inhibition of glucose transport in these carbohydrate-sensitive mutants would be protective at the level of the beta-cells. Changes in beta-cell granulation and islet cell volumes following DHEA feeding clearly demonstrated the protective effects on the endocrine pancreas of BL/Ks-*db/db* and *ob/ob* mice. This was identical to the effect of progesterone/estradiol injections reported previously.<sup>14</sup> Since estradiol<sup>27</sup> and hydrocortisone<sup>28</sup> block the induction of hyperglycemia in BL/Ks mice following multiple, low doses of streptozotocin, it is clear that adrenal and ovarian steroids are important modulators of beta-cell function and glycemic control in rodents. Since both cell-mediated and humoral immunity against islets has been reported in BL/Ks-*db/db* mice,<sup>29</sup> the pharmacologic doses of DHEA used may also be effecting therapy by immunosuppression of immunity against beta-cells.

Our previous studies with obese and diabetes mutants maintained on different inbred backgrounds have shown that the interaction of the mutant gene with background modifiers can have marked effects on the severity of the diabetes-obesity syndromes.<sup>12-14,19</sup> The present study suggests that the effectiveness of treatment with DHEA is also modulated by the inbred background of the host. Both the obese and diabetes mutations on either background produce gross metabolic abnormalities in a variety of pathways, so much so that it is difficult to evaluate either the mechanism of gene action itself or the action of any beneficial agents such as DHEA. The beneficial effects of DHEA exhibited in aging, disease-free mice suggest that the aging nonobese mouse might be a better model in which to evaluate the effects of DHEA.

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