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Levels of Prolactin, Growth Hormone and Insulin in Genetically Diabetic (db/db) Mice[1] (40494)

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The mutant diabetic (db/db) mouse was first described by Hummel *et al.* in 1966 (1). These mice suffer from metabolic disturbances resembling not insulin-dependent diabetes mellitus in man. The symptoms include hyperglycemia, polyuria, glycosuria and marked obesity. Like the obese mutant ob/ob, the db/db mice exhibit a paradoxical hyperglycemia concomitant with hyperinsulinemia in early stages of development (2). In later life, the insulin levels of db/db mice return towards normal, but the hyperglycemia persists. Glucose homeostasis under normal conditions is maintained by the interaction of several hormonal factors, which include insulin, growth hormone (GH), somatostatin and glucagon. Desjardins (3) found by bioassay that pituitary GH concentrations and GH-releasing factor (GH-RF) in the hypothalamus were both below normal in mildly hyperglycemic, 4- to 5 week-old db/db mice. In contrast, the two hormones were present at higher than normal levels in severely hyperglycemic, 12- to 13-week-old diabetics. However, the concentrations of GH in the sera of db/db mice have not been measured. In this study, we have determined the serum levels of immunoreactive GH, insulin, and prolactin (PRL), another pituitary hormone shown to influence glucose homeostasis (4), in normal and db/db mice of both sexes. The observations covered a period from 5 to 32 weeks of age.

Materials and methods. Mice used were of the C57BL/KsJ-db m strain, purchased from the Jackson Laboratory, Bar Harbor, Me. Twenty diabetic (db+/db+) and 20 related normal (db+/+m) male and 21 diabetic and 18 related normal female mice were obtained at 4 weeks of age. They were kept in 36 × 30 × 18 cm plastic boxes, 10 mice per box, in a light (12 hr light:12 hr darkness)- and temperature (24° ± 1°)-controlled room and given Wayne Lab Blox (6% fat, 24% protein, and 4.5% fiber) and water *ad libitum.*

Blood samples from each mouse were collected from the orbit at approximately 5, 7, 10, 15, 20, 24 and 28 weeks of age between 09:00 and 10:00 hr. In order to assess the secretory capacity of the hypothalamic–pituitary axis for PRL release, a PRL stimulation test with perphenazine (5) was performed in nine to ten female mice of each phenotype at 11 weeks of age. Finally, 4 weeks after the last bleeding, all surviving mice were decapitated within 30 sec after removal from the box, and the blood and pituitary glands were collected. All blood samples were centrifuged for serum separation within 2 hr of collection. The pituitary glands were weighed individually and extracted immediately with 0.05 M Na_2CO_3-$NaHCO_3$ buffer, pH 10, as described (6).

GH and PRL in all sera and pituitary extracts and insulin in a few samples in which enough serum was available were measured by specific radioimmunoassays (7–9). The biological potency of the mouse PRL standard used was 25 IU/mg and that of the mouse GH, 3.1 USP U/mg. Standard for the insulin assay was rat insulin, potency 20.7 IU/mg. Serum glucose was measured by the *o*-toluidine method (10). The results were analyzed by analysis of variance and Duncan's new multiple range test.

Results. Male and female db/db mice weighed approximately 35% more than controls at 5 weeks of age (Fig. 1). At 32 weeks, their body weights increased to over twice that of normal mice.

Serum glucose concentrations in db/db

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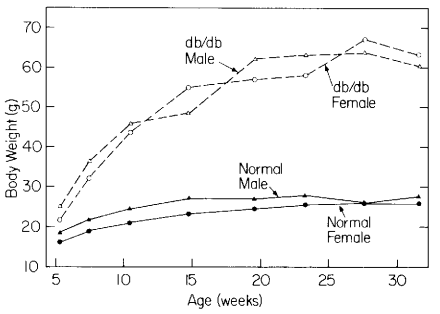

FIG. 1. Changes in body weight of normal and diabetic (db/db) mice with age. The numbers of mice in the various groups are those as shown in Fig. 5.

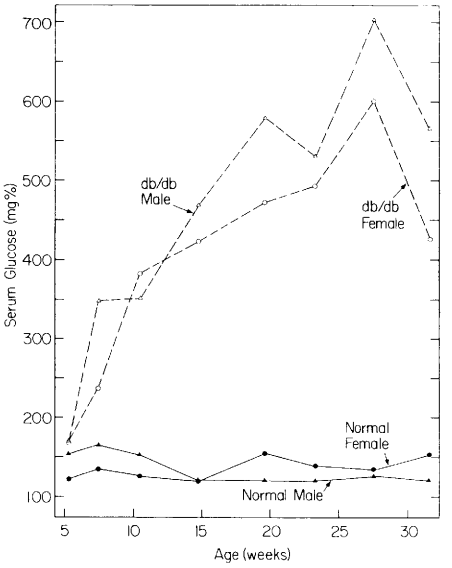

FIG. 2. Changes in blood sugar concentrations of normal and diabetic (db/db) mice with age. The numbers of mice in the various groups are those as shown in Fig. 5.

mice were only slightly elevated at 5 weeks (Fig. 2), but rose sharply thereafter and averaged 600–700 mg/100 ml at 28 weeks of age, as compared to only 130 mg/100 ml in control mice of the same age.

Serum insulin concentrations in db/db mice were higher than normal throughout the observation period (Fig. 3). At 32 weeks, insulin concentrations of db/db mice slightly declined.

GH concentrations in orbitally-collected sera of male db/db mice averaged markedly lower than those of normal male mice throughout the observation period ($P < 0.01$) (Fig. 4). In females, the differences were not statistically significant ($P > 0.05$). GH concentrations in the sera taken by decapitation from db/db mice of either sex at 32 weeks were also not appreciably different from those of controls ($P > 0.05$) (Table I). However, the pituitary concentrations of GH were substantially lower than normal in db/db mice of both sexes ($P < 0.01$).

PRL concentrations in orbitally collected sera showed a biphasic pattern in female db/db mice (Fig. 5): the levels were lower than

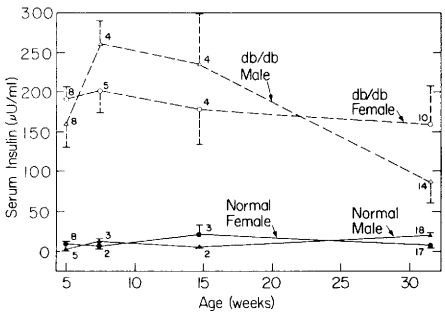

FIG. 3. Changes in serum insulin concentrations of normal and diabetic (db/db) mice with age. Vertical lines represent standard errors of the means. Numbers on the chart indicate the number of mice in each group; because of insufficient volume, only a few samples could be assayed for insulin. 20.7 μU = 1 ng.

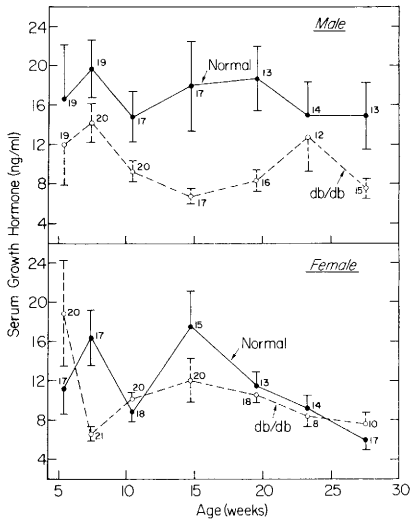

FIG. 4. Changes in growth hormone levels in the orbitally-collected serum of normal and diabetic (db/db) mice with age. Vertical lines represent SEM. Numbers on the chart indicate the number of mice in each group. The difference in the number of mice within a group at various intervals is due to insufficient sample or loss of animal by death.

TABLE I. BASAL LEVELS (MEAN + SEM) OF GROWTH HORMONE AND PROLACTIN IN DECAPITATE SERA AND PITUITARY GLANDS OF 32-WEEK-OLD DIABETIC (db/db) MICE.

Sex	Phenotype	No. of mice	Body wt (g)	Pituitary gland wt (mg)	Growth Hormone		Prolactin	
					Serum (ng/ml)	Pituitary (μg/mg)	Serum (ng/ml)	Pituitary (μg/mg)
Male	Normal	16	28 ± 0.8	1.61 ± 0.04	8.3 ± 1.0	76.1 ± 4.6	30.4 ± 1.7	3.6 ± 0.14
	Diabetic	14	60 ± 2.0**	1.52 ± 0.04	7.3 ± 0.4	49.9 ± 1.8**	24.6 ± 1.1*	2.4 ± 0.07**
Female	Normal	18	26 ± 0.4	2.13 ± 0.08	9.2 ± 0.9	82.2 ± 4.1	76.0 ± 13.7	8.7 ± 0.58
	Diabetic	10	59 ± 3.0**	1.50 ± 0.04**	7.8 ± 1.5	51.8 ± 2.3**	33.2 ± 1.3**	2.9 ± 0.28**

* $P < 0.05$, ** $P < 0.01$; comparisons shown are between the two phenotypes of the same sex.

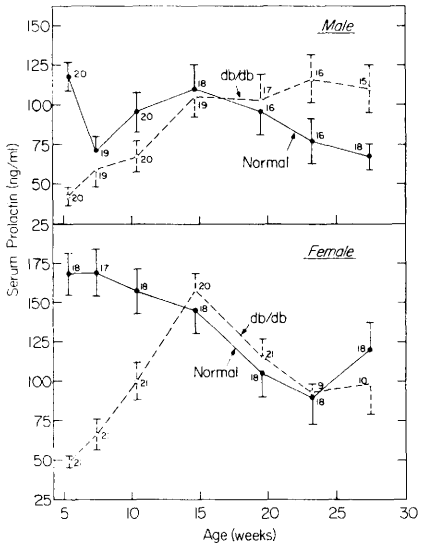

FIG. 5. Changes in prolactin levels in the orbitally-collected serum of normal and diabetic (db/db) mice with age. Vertical lines represent standard errors of the means. Numbers on the chart indicate the number of mice in each group. The difference in the number of mice within a group at various intervals is due to insufficient sample or loss of animal by death.

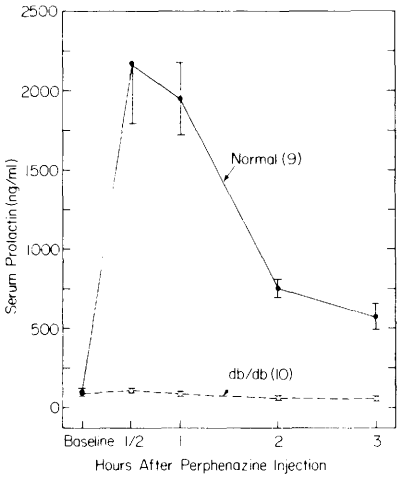

FIG. 6. Serum prolactin concentrations in normal and diabetic (db/db) female mice after stimulation with perphenazine (1 μg/g body wt). Vertical lines represent SEM. Values in parentheses indicate the number of mice in each group.

normal during early stages ($P < 0.01$), i.e., until about 10–12 weeks of age; then the levels increased to those found in normal mice ($P > 0.05$). In males, the trend was similar, but the differences were less pronounced. PRL concentrations in sera collected after decapitation at 32 weeks were significantly lower in db/db mice of both sexes ($P < 0.01$), as were the pituitary concentrations ($P < 0.01$) (Table I). However, the magnitude of the differences was less pronounced in males.

A single ip injection of 1 μg/g BW of perphenazine elicited a 20-fold rise in serum PRL of female control mice ($P < 0.01$) (Fig. 6). There were no increases in PRL concentrations of female db/db mice.

Discussion. These studies indicate that the db/db mouse has significantly altered secretory patterns of GH and PRL. The consistently lower GH levels in the orbitally collected serum of male db/db mice, in conjunction with lower glandular content of the hormone in db/db mice of both sexes, suggest that the secretion of GH is most likely impaired in this mutant. Our results on pituitary GH content differ from the bioassay results of Desjardins (3), who found higher levels of GH and GH-RF in 12- to 13-week-old db/db mice. This discrepancy between the two studies may be due to the fact that different assays were used. However, Desjardins (3) also found lower than normal levels of pituitary GH in 4- to 5-week-old db/db mice. We did not measure pituitary GH at this age, but our measurements in sera from animals at 5 to 32 weeks of age show no clear age-related shifts in GH concentrations. The GH concen-

trations in the orbitally collected sera of female db/db mice were not greatly diminished, but female mice generally have low GH activity (11) and thus are subject to less pronounced fluctuations. The similar GH concentrations in sera obtained at 32 weeks from decapitated db/db and control mice were perhaps caused by the animals' age, since Larson *et al.* (12) found that the earlier differences in the GH concentrations of ob/ob mice disappeared when the mice reached 20 weeks of age.

The secretion of PRL in db/db mice was also, for the most part, deficient, as indicated by the levels of the hormone in the pituitary gland, in sera obtained by decapitation, and by the lack of PRL release in response to perphenazine. In orbitally collected sera, PRL concentrations were below normal before 15 weeks, but normal afterwards. The concentration of PRL in orbitally collected serum is usually higher than in serum collected by decapitation (8). Thus, it seems that during early stages, db/db mice did not react as much to the stress of orbital bleeding as did normal mice, whereas after 15 weeks they reacted more than the normals. The reason for this biphasic response is not clear, but it is noteworthy that the serum insulin levels in our study (Fig. 3) and those of others (2, 13) also show a shift towards normal with age.

The differences from normal in GH, PRL, and insulin levels of db/db mice were evident at 5 weeks of age, the first time when observations were made. At this age, the hyperglycemia and obesity were still quite moderate. This suggests that the sub-normal levels of GH and PRL found in db/db mice are probably the result of mutational changes in the background genome rather than a consequence of the obesity–diabetes syndrome. Similar differences in GH and PRL preceding the appearance of obesity are found in ob/ob mice (12). Thus, the GH and PRL abnormality may represent a common secondary gene action of the mutation db and ob. To what extent the reduced levels of GH and PRL contribute to the development or exacerbation of the obesity–diabetes syndrome in db/db mice is not certain.

Summary. Levels of GH, PRL, and insulin in diabetic (db/db) mice were studied by radioimmunoassay. Serial blood samples from normal and diabetic mice of both sexes were obtained by orbital puncture at 5, 7, 10, 15, 20, 24, and 28 weeks of age; the final sample was obtained by decapitation at 32 weeks. Serum insulin and glucose levels in db/db mice were higher than normal throughout the study. Compared to those in normal controls, GH and PRL concentrations in the pituitary gland were 33 to 67% lower in db/db mice at 32 weeks of age. Serum GH levels in orbitally collected samples were also markedly subnormal in male diabetics; in females, the differences were not significant. GH levels in sera collected at 32 weeks were not significantly different from those of controls in either sex. PRL concentrations in the orbitally collected sera of db/db mice were lower than normal until 10–12 weeks of age; after this age the values approached normal levels. PRL concentrations in sera collected at decapitation were significantly lower than normal in db/db mice of both sexes. A single injection of perphenazine elicited a 20-fold rise in serum PRL concentrations in normal females; in db/db females, there were no increases. The results suggest generally deficient secretion of GH and PRL in the mutant db/db mouse.

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