

Diminished Hepatic Growth Hormone Receptor Binding in Sex-Linked Dwarf Broiler and Leghorn Chickens (42473)

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*Abstract.* Hepatic growth hormone (GH) receptor binding was compared in normal and sex-linked dwarfs (SLD) from both Hubbard and Cornell strain chickens. At 6, 8, and 20 weeks of age, hepatic GH receptor binding in the Hubbard SLD chickens was significantly lower than that of normal fast-growing birds. At 20 weeks of age, only 2 of 22 SLD chickens in the Hubbard broiler strain showed positive binding at a high enough level to allow for Scatchard analysis. The affinity constants and binding capacities of these two SLD chickens were numerically (but not significantly) lower than those of the normal fast-growing birds. We further examined hepatic GH receptor binding in two closely related White Leghorn strains of chickens that have been maintained as closed breeding populations for many years. We observed no detectable hepatic GH binding in the Cornell SLD chickens ( $N = 20$ ), as compared to the normal-growing control strain (K strain). In both SLD strains, pretreatment with 4 M MgCl<sub>2</sub> did not enhance GH binding, suggesting that there was no endogenous GH binding to the receptor. Based on these data, we suggest that the lack, or greatly reduced number, of GH receptors may be a major contributing factor to the dwarfism observed in these strains. © 1987 Society for Experimental Biology and Medicine.

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The hormone profile of chickens carrying the sex-linked dwarfing gene has been well characterized (1-3). Sex-linked dwarf (SLD) chickens have significantly depressed circulating T<sub>3</sub> levels and marginal T<sub>4</sub> levels, which suggests that these chickens are functionally hypothyroid. Paradoxically, growth hormone (GH) synthesis and immunoactive circulating GH concentrations were higher in SLD chickens than in normally growing birds. In spite of the high circulating GH concentrations, serum somatomedinlike factors also have been found lower in both the layer and the broiler SLD strains (4, 5).

Recently, we have identified and characterized specific hepatic receptors for chicken GH (cGH) and have developed a homologous radioimmunoassay (6, 7). We now report the results of comparing hepatic GH receptor binding in SLD of both the fast-growing broiler and the Leghorn strain chickens. We suggest that the dwarfism found in SLD may be due,

at least in part, to the lack of GH receptors or the reduced numbers of GH receptors.

**Materials and Methods.** *Animals.* Fast-growing and SLD broilers were obtained from Hubbard Farms (Walpole, NH); normal-growing (K strain) and SLD White Leghorn chickens were obtained from Cornell University (Ithaca, NY). The two Leghorn strains are closely related and have been maintained as closed breeding populations for many years (at least 20) (8, 9). The SLD broiler and Leghorn chickens both carry the same dwarfing gene, first described by Hutt (8). The effects of the dwarfing gene on circulating hormone concentrations and body weight gain were reported to be similar in both the broiler and the Leghorn chickens (1, 2). The broiler chickens are fast-growing, meat-type birds, and the Leghorns are slow-growing, layer-type birds. All birds were housed at 25°C in a light-regulated (14-hr light/10-hr dark) environment. At all times, feed and water were available *ad libitum*. Chickens were killed by decapitation, and livers were immediately frozen at -80°C for GH receptor isolation.

*cGH binding assay.* A purified pituitary preparation of cGH was iodinated by a lac-

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toperoxidase method previously described by us (6). Specific radioactivity of  $^{125}\text{I}$ -labeled cGH was generally between 80 and 120  $\mu\text{Ci}/\mu\text{g}$ . Membrane fractions were prepared from individual livers. Microsomal fractions (100,000g), prepared as described by Shiu *et al.* (10), were lyophilized for storage. These microsomal fractions were homogenized in Tris-HCl assay buffer (0.025 M; pH 7.6) containing 0.5% bovine serum albumin when required for assay. For the receptor assay, 600  $\mu\text{g}$  of membrane protein, as estimated by Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, CA) were added to a test tube containing 100  $\mu\text{l}$  of assay buffer, 100  $\mu\text{l}$  of [ $^{125}\text{I}$ ]cGH ( $5 \times 10^4$  cpm), and 100  $\mu\text{l}$  of assay buffer with or without excess unlabeled cGH. Specific binding of cGH was determined by incubating [ $^{125}\text{I}$ ]cGH with the membrane fractions, either in the presence or in the absence of 1  $\mu\text{g}/\text{tube}$  of unlabeled hormone. Nonspecific binding in all membrane preparations tested was 8 to 10% of the total radioactivity added. After incubation at room temperature for 16 to 20 hr, 1 ml of cold buffer was added to the contents of each tube, after which tubes were centrifuged at  $4^\circ\text{C}$  for 10 min at 100g. The supernatant was decanted, and the bound [ $^{125}\text{I}$ ]cGH was counted in an automatic  $\gamma$  counter (Micromedic Model 4/600; Micromedic Systems, Inc., Horsham, PA). The apparent dissociation constant and GH binding capacity were determined by Scatchard analysis (11). Scatchard analyses were done by incubating a fixed amount of membrane protein with a fixed amount of labeled hormone and increasing amounts of unlabeled standard.

To test for possible occupancy of receptors by endogenous hormone, receptor bindings in membranes prepared from livers of normal and SLD chickens (both Hubbard broiler and Cornell Leghorn) were tested with pretreatment of 4 M  $\text{MgCl}_2$ . It has been reported that removal of endogenous bound ligand can be achieved by treatment with 4 M  $\text{MgCl}_2$  (12-14).

Statistical differences in receptor cGH binding between the SLD and their respective control strains were analyzed by Student's *t* test and  $\chi^2$  analysis.

**Results.** The specific hepatic GH receptor binding in normal fast-growing and SLD

broilers (Hubbard strain) is shown in Fig. 1. The specific cGH binding of the fast-growing broilers was low at 6 weeks of age (<1.0%) and increased to 17.0% at 20 weeks of age. The specific cGH binding by the SLD liver membranes was also low at 6 weeks of age and remained low through 20 weeks of age (<1.0%). At all ages studied, the specific hepatic cGH receptor binding was significantly lower in the SLD than in the fast-growing broilers;  $\chi^2$  analysis of the frequency data also revealed a significant difference between the two genotypes. There were no significant differences observed in specific cGH receptor binding between 4 M  $\text{MgCl}_2$ -treated and nontreated membranes from the 8-week-old, fast-growing and SLD broiler chickens (Table I). A similar lack of response to 4 M  $\text{MgCl}_2$  was observed in Leghorn chickens (data not shown).

Binding affinity constant and binding capacity were determined by Scatchard analysis. Of the four "atypical" 20-week-old SLD broiler liver samples that showed positive specific GH binding, only two had high enough binding to allow Scatchard analysis (Fig. 2).

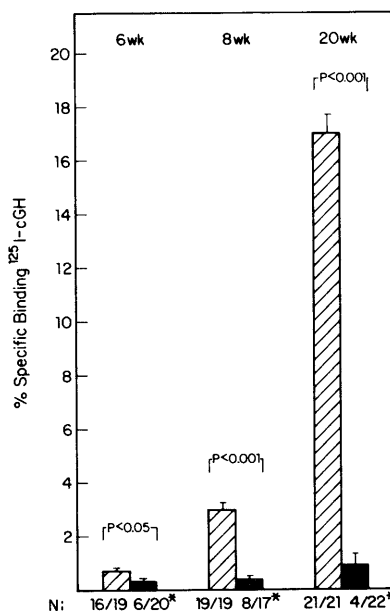


FIG. 1. Specific hepatic growth hormone (GH) receptor binding in normal fast-growing (hatched bars) and sex-linked dwarf broiler male chickens (solid bars) at 6, 8, and 20 weeks of age. *N* = number of preparations, showing positive binding/number of total preparations; \* $P < 0.01$ , compared with normal ( $\chi^2$  analysis).

TABLE I. EFFECTS OF 4 M MgCl<sub>2</sub> ON SPECIFIC HEPATIC GROWTH HORMONE RECEPTOR BINDING IN 8-WEEK-OLD, NORMAL FAST-GROWING AND SEX-LINKED DWARF BROILER CHICKENS

Strain	Percent specific binding $\pm$ SEM	Number positive/total	MgCl <sub>2</sub> treated	
			Percent specific binding $\pm$ SEM	Number positive/total
Normal	2.94 $\pm$ 0.25	19/19	2.81 $\pm$ 0.28	19/19
Dwarf	0.40 $\pm$ 0.08 <sup>a</sup>	08/17 <sup>b</sup>	0.50 $\pm$ 0.15 <sup>a</sup>	06/17 <sup>b</sup>

<sup>a</sup>  $P < 0.001$ , compared with normal chickens; Student's  $t$  test.

<sup>b</sup>  $P < 0.01$ , compared with normal chickens;  $\chi^2$  analysis.

The respective affinity constants obtained from the fast-growing and SLD broilers at 20 weeks of age were  $8.4 \pm 0.01$  (SE)  $\times 10^9 M^{-1}$  ( $N = 11$ ) versus  $3.9 \pm 0.01 \times 10^9 M^{-1}$  ( $N = 2$ ). The binding capacities of the two strains were  $11.23 \pm 1.76$  fmole/mg ( $N = 11$ ) versus  $6.04 \pm 1.70$  fmole/mg ( $N = 2$ ), respectively. The apparent decrease in affinity constants and binding sites of GH receptors at 20 weeks in the two SLD broiler chickens that showed minimal binding ( $<1\%$ ) was not significantly

different from those of normal, fast-growing chickens. No binding of cGH was detectable in the remaining 18 dwarf broiler chickens examined.

The specific hepatic GH receptor binding data obtained from normally growing and SLD Leghorn chickens at 20 weeks of age is shown in Table II. There was no detectable GH binding in the SLD Leghorn chickens ( $N = 20$ ). Specific receptor binding GH was detected in both male and female normally growing chickens, and there were significantly fewer GH receptors in female than in male chickens.

**Discussion.** The results of this study clearly indicate that the hepatic GH receptor binding capacity is markedly reduced in SLD chickens of both Hubbard and Leghorn strains. The virtual absence of cGH receptor binding offers a plausible explanation for the poor growth of chickens carrying the SLD mutant gene. The Cornell Leghorn strain is more closely inbred than the Hubbard broiler strain, and the expression of the SLD gene is dependent on the background genotype of the chicken (15). Since the SLD mutant gene is fixed in both Cornell and Hubbard strains, it is likely that the heterogeneity of background genes in the Hubbard SLD chicken is responsible for the minimal amount of GH binding noted in four "atypical" lines.

Circulating GH concentrations have been reported to be significantly higher in SLD than in normal age-matched chickens (1-4). A possible interpretation of the inverse relationship between serum GH concentrations and GH receptor binding capacity is that the excess circulating GH may be down-regulating its receptor. On the contrary, rat GH has been shown to induce (up-regulate) its own hepatic receptor in rats (13). Information regarding

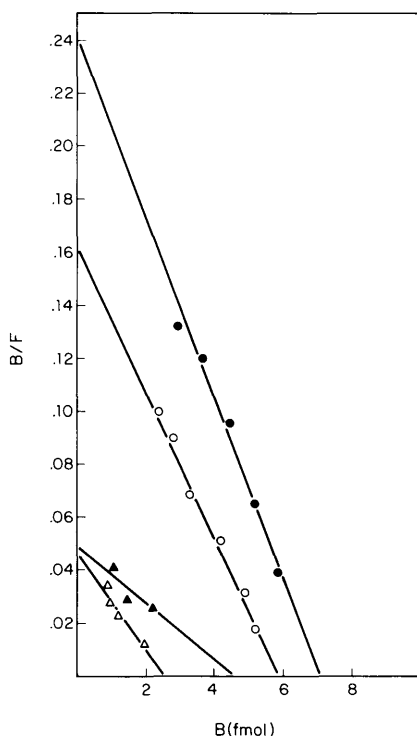


FIG. 2. Scatchard analysis of two representative 20-week-old, fast-growing (○, ●) and two "atypical" sex-linked dwarf (△, ▲) broiler chickens.

TABLE II. HEPATIC GROWTH HORMONE RECEPTOR BINDING DATA IN 20-WEEK-OLD, NORMALLY GROWING AND SEX-LINKED DWARF (SLD) LEGHORN CHICKENS<sup>a</sup>

Strain	Sex	Percentage specific binding $\pm$ SEM	Number positive/total
Normally growing	Male	11.45 $\pm$ 0.66	10/10
	Female	5.10 $\pm$ 0.60 <sup>b</sup>	09/09
SLD	Male	NB <sup>c</sup>	0/09
	Female	NB	0/11

<sup>a</sup>  $P < 0.01$ , compared with normal chickens;  $\chi^2$  analysis.

<sup>b</sup>  $P < 0.01$ , compared with male chickens; Student's  $t$  test.

<sup>c</sup> No binding detected.

the regulation of GH receptor in other species is lacking. If the hypothesis that GH down-regulates its own receptor in chickens is correct, this suggests that the SLD mutant gene acts at the level of the pituitary (GH synthesis and release). Alternatively, the inverse relationship may result from a GH receptor-related reduction in negative feedback control of pituitary GH secretion. This suggests that the SLD mutant gene acts at the level of the GH receptor by decreasing the number of GH receptors or by altering the GH binding domain of the receptor. The low serum somatomedin levels found in SLD chickens support the hypothesis that GH receptor-mediated functions in SLD livers are reduced (4, 16). Our attempt to determine if SLD hepatic GH receptors were totally bound by endogenous cGH through pretreatment with 4 M MgCl<sub>2</sub> did not support the occupancy argument. However, further study of the regulation of GH receptor in chickens and its occupancy to the receptor are important to delineate SLD mutant gene action.

The increase of hepatic cGH receptor binding with age in the normal chickens confirms our previous findings (6). High circulating GH concentrations in young chickens and decreased concentrations with age in normal chickens also are well documented (17). Thus, it appears that the combined analysis of serum GH concentrations and hepatic GH receptor binding has relevance for growth studies in normal chickens. Furthermore, the Cornell Leghorn chickens grew more slowly than the

Hubbard chickens, and the Cornell chickens showed less GH receptor binding than the Hubbard chickens. Apparently, there is a better correlation between GH binding and growth in chickens than between circulating GH concentrations and growth.

It is interesting to compare the serum GH profile and receptor binding data relative to dwarfism in chickens and mice. The dwarf mouse models (Little, Snell, and Ames dwarfs) have serum GH deficiencies. Despite a similar deficiency in GH, the deficiencies are heterogeneous. The Little mice have isolated GH deficiencies, while the Snell and Ames mice have deficiencies in GH as well as in other anterior pituitary hormones. In addition, it was recently demonstrated that mGH mRNA in the dwarf mice is deficient (18). Furthermore, the Little mice have normal GH receptors, whereas GH receptor binding is decreased in Snell dwarf mice (19). Each mouse model has low circulating somatomedin concentrations, as do the SLD chickens (16, 20). Despite the similarities, the mouse mutant genes differentially affect the physiology of GH (18, 21). At this time, however, it seems plausible that low hepatic cGH binding and low somatomedin production contribute significantly to the SLD phenotype and that SLD mutant gene action has a detrimental effect on GH receptors. Additional studies are needed to assess the validity of the SLD mutant gene-induced GH receptor defect.

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