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Chronic Administration of Growth Hormone (GH) to Adult Chickens Exerts Marked Effects on Circulating Concentrations of Insulin-Like Growth Factor-I (IGF-I), IGF Binding Proteins, Hepatic GH Regulated Gene I, and Hepatic GH Receptor mRNA

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In young birds, growth hormone (GH) administration has been found to have only a small or even no effect on circulating concentrations of insulin-like growth factor-I (IGF-I). This is in obvious contrast to the situation in mammals. The present study examines the effect of continuous administration of GH in adult male chickens. Plasma concentrations of IGF-I were markedly elevated (2.5–3.0-fold, $p < 0.001$) in GH-treated chickens. There were also some transient increases in the circulating levels of IGF binding proteins. Adult chickens showed other manifestations of increased responsiveness to GH, including elevated hepatic expression of GH-regulated gene-I (mRNA) with GH treatment ($p < 0.05$), and a tendency ($p < 0.08$) for decreased GH-receptor mRNA. In contrast to the changes in circulating concentrations of GH and IGF-I with GH treatment, no changes in plasma concentrations of thyroid hormones, reproductive hormones, glucose, or nonesterified fatty acids were evident.

Key Words: Growth hormone (GH); chicken; insulin-like growth factor-I (IGF-I); IGF binding proteins; GH receptor.

Introduction

In postnatal mammals, circulating concentrations of insulin-like growth factor-I (IGF-I) are maintained by growth hormone (GH) acting predominantly on the liver. Approximately 95% of circulating concentrations of IGF-I appears to be dependent on adenohypophyseal hormones, based on the reductions in circulating concentrations of IGF-I occurring following hypophysectomy in rats *(1,2)* or in GH-deficient dwarf mice *(3)*. Based on replacement therapy studies, GH is the major (if not the only) pituitary hormone controlling circulating concentrations of IGF-I. If additional GH is present in intact animals (because of, for instance, the administration of exogenous GH), circulating concentrations of IGF-I can be further elevated. In livestock species, circulating concentrations of IGF-I are elevated by the chronic administration of GH (e.g., pigs: *see* refs. *4* and *5*; lambs: *see* ref. *6*; and dairy cattle: *see* ref. *7*).

The response in poultry species to GH depletion or administration is both quantitatively and qualitatively different from mammals. In young growing birds, hypophysectomy reduces circulating concentrations of IGF-I by only about 50%, with GH-replacement therapy increasing circulating concentrations by only about 20% (chicken: *see* ref. *8* and turkey: *see* ref. *9*). In intact growing chickens, GH treatment results in either no change *(10)* or only small increases in circulating concentrations of IGF-I *(11)*. Similarly in young turkeys, GH treatment has only a small (~20%) stimulatory effect on plasma concentrations of IGF-I *(12)*.

The present study examines the ability of GH to elevate circulating concentrations of IGF-I and to influence the expression of GH-regulated gene-I (GHRG-I) mRNA and GH-receptor mRNA in adult male chickens. It should be noted that GHRG-I was first identified based on its expression in normal, but not in GH-receptor deficient sex-linked dwarf chickens *(13)*. It is hypothesized that adult chickens with their low circulating concentrations of GH *(14)* and IGF-I *(15)* would be very responsive to GH, as seen in hypophysectomized rodents. Moreover, hepatic GH-recep-

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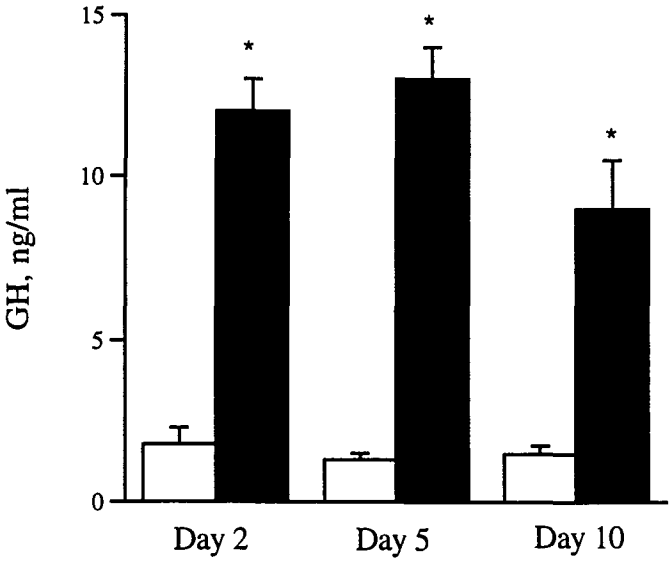

Fig. 1. Effect of chronic continuous administration of GH on plasma concentrations of GH in adult male chickens. Each bar represents pooled values from study 1 and 2, ±SEM (n = 8–9). Open bars, control; solid bars, GH. *Control vs GH, $p < 0.001$.

tor levels (specific binding of ^{125}I-GH) are much higher in adult as compared to young birds *(16,17)* as are the levels of the GH-receptor mRNA in the liver *(15)*. This again mitigates for a greater responsiveness to GH in the adult chicken. In addition, the high level of hepatic GH receptor facilitates examination of whether GH can downregulate its receptors, which was also examined in this study. Finally, the chronic administration of GH via osmotic pump allowed the determination of chronic effects of GH on circulating IGF-binding proteins (IGFBP), metabolites, thyroid, and reproductive hormones and organ weights in adult male chickens.

Results

As expected, infusion of recombinant chicken growth hormone (rcGH) by osmotic minipumps increased ($p < 0.001$) circulating concentrations of GH (interaction with time, $p < 0.001$) in adult male chickens (Fig. 1). Within 2 d, circulating concentrations of GH were increased by approx six-fold and this was maintained throughout the treatment period.

Body and Organ Weights, Plasma Metabolites, and Hormones

Infusion of rcGH decreased ($p < 0.05$) the weight of the abdominal fat pad (interaction with time, $p > 0.2$) after 10 d of treatment compared to controls (Table 1). This may reflect GH preventing the increase in adipose weight, which occurred posttransfer to individual cages. Body, breast muscle, liver, spleen, thymus, and testis weights were not affected by rcGH infusion (Table 1). Although circulating concentrations of T_3 were not affected by GH, after 10 d T_4 was greater and the T_3/T_4 ratio lower ($p < 0.05$) (Table 2) in GH-treated chickens. Circulating concentrations of luteinizing hormone (LH) and prolactin were not affected by rcGH infusion (Table 2). Similarly, no changes were seen in circulating concentrations of nonesterified fatty acids (NEFAs) or glucose (Table 2) with rcGH infusion.

IGF-I and IGFBP

Concomitant with the elevation in circulating concentrations of GH in GH-treated chickens, there were marked increases ($p < 0.001$) in circulating concentrations of IGF-I (interaction with time, $p < 0.01$) (Fig. 2A). Circulating concentrations of IGF-I were 2.5- (d 2), 3- (d 5), and 2.8- (d 10) fold greater in GH-treated chickens as compared to controls.

Some increases in plasma levels of IGFBPs were also observed with GH infusion. The 22 kDa IGFBP (Fig. 2B) was increased ($p < 0.05$) on d 2 in GH-treated adult male chickens. This response did not continue throughout the treatment period, as no differences were observed between treatments on d 5 and 10. Infusion of rcGH tended to increase ($p < 0.08$) and increased ($p < 0.05$) the plasma level of the 28 kDa IGFBP on d 2 and 5 of treatment, respectively. As seen for the 22 kDa IGFBP, this response was not evident at d 10 (Fig. 2C). The plasma level of the 36 kDa IGFBP was also elevated ($p < 0.05$) by rcGH infusion on d 2. This effect was not seen on d 5, but was again evident on d 10 ($p < 0.05$) (Fig. 2D).

GHR mRNA/GHRG-I/GHR

Infusion of adult chickens with rcGH increased ($p < 0.05$) the level of hepatic GHRG-I mRNA on d 2 and 5 of study 2 by, respectively, 6.1- and 2.7-fold (Fig. 3A,C). Day 10 samples were not analyzed. The level of GHR mRNA was not affected by treatment after 2 d, but tended ($p < 0.08$) to be decreased with GH infusion (54% of control) after 5 d of treatment (Fig. 3B,D). Day 10 samples were not analyzed.

Discussion

Growth hormone evokes large increases in circulating concentrations of IGF-I both in GH-deficient mammals (e.g., hypophysectomized rats: *see* refs. *2* and *18*; dwarf mice: *see* ref. *3*) and intact growing mammals (e.g., pigs: *see* refs. *4*, *5*, *19*, and *20*; lambs: *see* refs. *6* and *7*). An exception to this may be growing cattle where only modest increases in circulating concentrations of IGF-I are reported *(21,22)*. However, large increases in circulating concentrations of IGF-I are observed in adult cattle (e.g., *see* ref. *23*). Previously, GH has been observed to evoke only small increases (<< 50%) in the circulating concentrations of IGF-I in hypophysectomized young chickens *(8)* and intact chickens and turkeys *(9,11,12)*. However, in the present study with adult male chickens, GH induced large (2–3.5-fold) increases in the circulating concentrations of IGF-I. In view of the high number of GH receptors [^{125}I-GH binding sites and GH-receptor mRNA *(15)*] in the liver of adult chickens and the low circulating concentrations of GH (e.g., *see* ref. *14*), it was originally postulated that the

Table 1
Effect of Recombinant Chicken GH (100 mg/d from s.c. osmotic pump) on Body and Organ Weights in Adult Male Chickens (means ± SEM, n = 4–5): Study 2

	Day 2		Day 5		Day 10	
Item	Vehicle	GH	Vehicle	GH	Vehicle	GH
Body wt, kg	2.1 ± .2	2.1 ± .1	2.0 ± .03	1.9 ± .1	2.4 ± .1	2.3 ± .03
Breast muscle, g	89.8 ± 5.0	88.5 ± 8.5	81.8 ± 3.5	86.7 ± 4.6	109.2 ± 2.4	99.8 ± 5.3
Abdominal fat, g	11.1 ± 3.0	13.1 ± 6.7	17.0 ± 7.4	5.7 ± 2.5	25.9 ± 3.5[a]	10.1 ± 3.2[b]
Liver, g	39.3 ± 3.1	39.4 ± 1.9	33.6 ± 1.6	33.1 ± 3.0	42.0 ± 5.3	39.7 ± 2.1
Spleen, g	3.4 ± .51	3.1 ± .19	2.7 ± .28	2.9 ± .44	4.0 ± .51	3.6 ± .41
Thymus, g	.66 ± .08	.44 ± .11	.24 ± .05	.30 ± .06	.63 ± .23	1.57 ± .93
Testis, g	ND	ND	ND	ND	13.1 ± 1.8	15.7 ± 2.1

[ab]GH effect on abdominal fat pad weight on d 10, $p < 0.05$.
ND, no data.

Table 2
Effect of Recombinant Chicken GH (100 mg/d from s.c. osmotic pump) on Circulating Concentrations of Glucose, Nonesterified Fatty Acids (NEFAs), Luteinizing Hormone (LH), Prolactin, Follicle-Stimulating Hormone (FSH), and Thyroid Hormones in Adult Male Chickens (means ± SEM, n = 4–5)

	Day 2		Day 5		Day 10	
Item	Vehicle	GH	Vehicle	GH	Vehicle	GH
Glucose, mg/dL	217 ± 22	206 ± 9.5	232 ± 11	218 ± 9.6	247 ± 5.1	229 ± 9.1
NEFA, mEq/L	301 ± 38	312 ± 36	208 ± 27	238 ± 20	191 ± 14	224 ± 16
LH, ng/mL	3.1 ± .92	2.0 ± .47	2.4 ± 1.1	1.5 ± .12	2.0 ± .72	2.5 ± 2.0
Prolactin, ng/mL	7.5 ± 1.1	8.7 ± .8	6.1 ± 1	5.8 ± 1	3.1 ± .8	2.4 ± .1
FSH, ng/mL	4.3 ± .9	2.9 ± 1.4	4.7 ± 2.6	2.9 ± .6	3.2 ± 1.2	.67 ± .5
T_3, ng/mL	2.3 ± .13	2.6 ± .28	2.4 ± .39	2.6 ± .52	2.9 ± .25	2.0 ± .83
T_4, ng/mL	8 ± 0.3	8 ± 0.3	10 ± 0.9	12 ± 1.3	8 ± 0.4[a]	11 ± 1[b]
T_3/T_4 ratio	.29 ± 0.02	.34 ± 0.04	.26 ± 0.07	.26 ± 0.07	.33 ± 0.02[a]	.19 ± 0.04[b]

[ab]Vehicle vs GH are different on d 10, $p < 0.05$.
Glucose values are from study 1. All other values from study 2.

adult chicken would be much more responsive to exogenous GH as compared to the well-established situation in young chicks. This was, indeed, the case.

Continuous administration of GH to adult chickens (and subsequent increases in IGF-I) was accompanied by increases in circulating concentrations of IGFBPs. Although the elevated plasma concentrations of the 22 kDa IGFBP was short lived, the 36 kDa IGFBP (and to a lesser extent the 28 kDa IGFBP) remained elevated through this study. The observed increase in IGFBPs is in agreement with observations in other species where GH has been administered *(2,3,19,24)*. The increase in IGFBPs (IGFBP3) associated with GH administration in these previous studies is maintained throughout the trial. The increase in circulating levels of the 28 and 36 kDa IGFBPs in the present study suggest these IGFBPs may be homologous to the mammalian IGFBP3 doublet, which seems to be GH dependent.

In the present study, GH elevated GHRG-I expression. This provides strong support for the view that expression of GHRG-I is GH-dependent *(13)*. The original identification of GHRG-I expression as GH-dependent rested on presence of GHRG-I mRNA in control, but not GH-receptor deficient sex-linked dwarf chickens, parallel ontogenic changes in circulating concentrations of GH and hepatic GHRG-I expression in control chickens, and similarity between the promoter for the GHRG-I and the GH response element in the serum protease inhibitor gene, Spi 2.1 *(13)*. The observed increases in hepatic GHRG-I mRNA levels in GH-treated chickens provide direct evidence for GHRG-I expression being under GH control. This is again consistent with the view that the adult chicken is a very useful model for GH action with its low endogenous circulating concentrations of GH and high GHR expression.

Administration of GH tended to reduce the levels of hepatic GHR mRNA. This is consistent with GH down-regulating GHR availability, partially, but not completely by reducing GHR expression. During growth and development, there are inverse relationships between circulating concentrations of GH and hepatic ^{125}I-GH binding *(16,17)* or GHR mRNA *(15)*. Moreover, hypophysectomy of young chickens

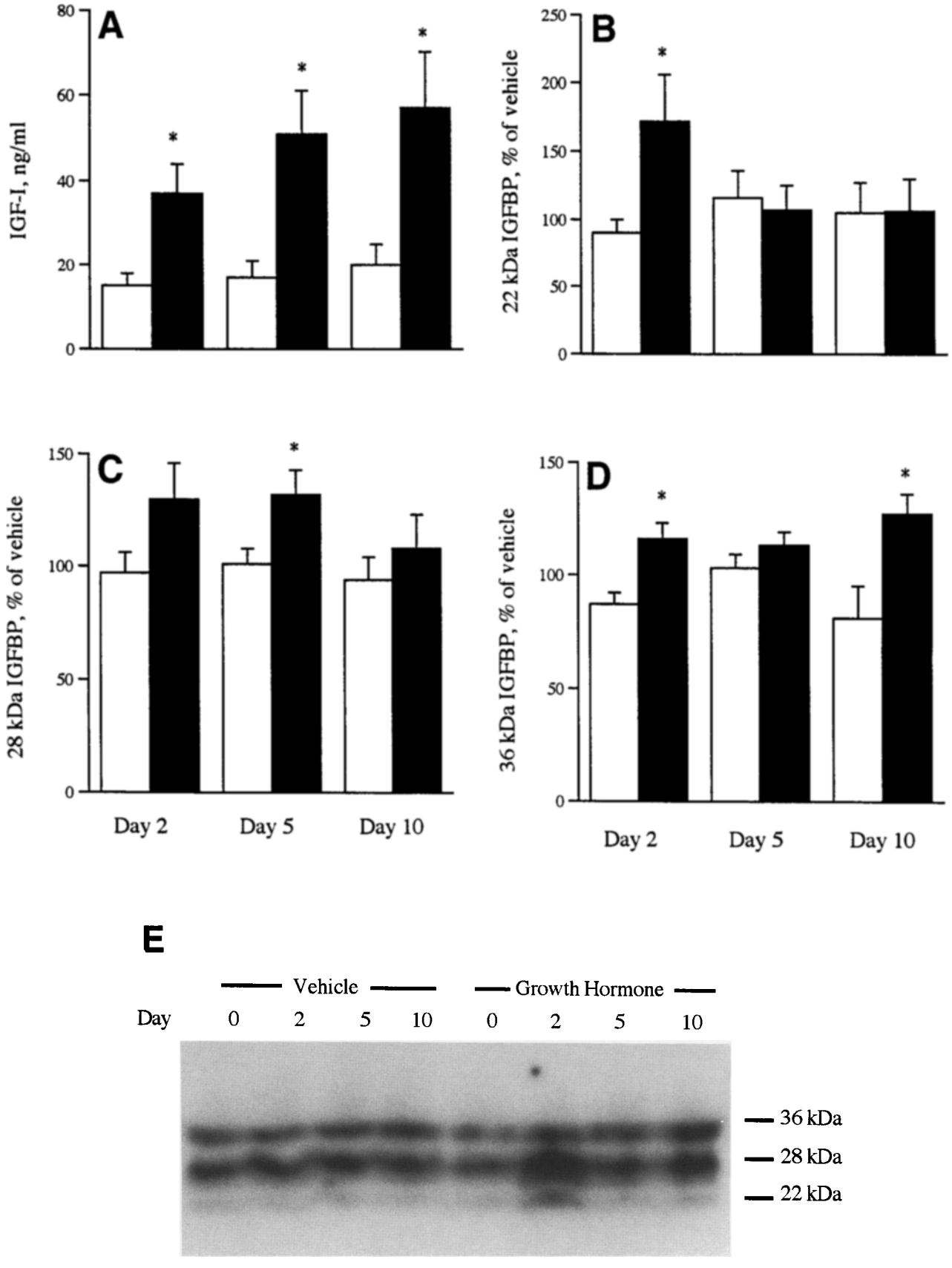

Fig. 2. Effect of continuous administration of GH on plasma concentrations of IGF-I and IGFBPs in adult male chickens. Plasma concentrations of **(A)** IGF-I and **(B–D)** IGFBPs are presented for d 2, 5, and 10 of treatment with control (open bars, unimplanted or saline implanted) or GH (solid bars). IGFBPs are expressed as a percentage of vehicle-infused chickens (B) 22 kDa, (C) 28 kDa, and (D) 36 kDa. Each bar represents the mean ±SEM, with $n = 8–9$. **(E)** Representative IGFBP radioligand blot, molecular weights are indicated. Plasma from study 1. *Vehicle vs GH, within day, $p < 0.05$.

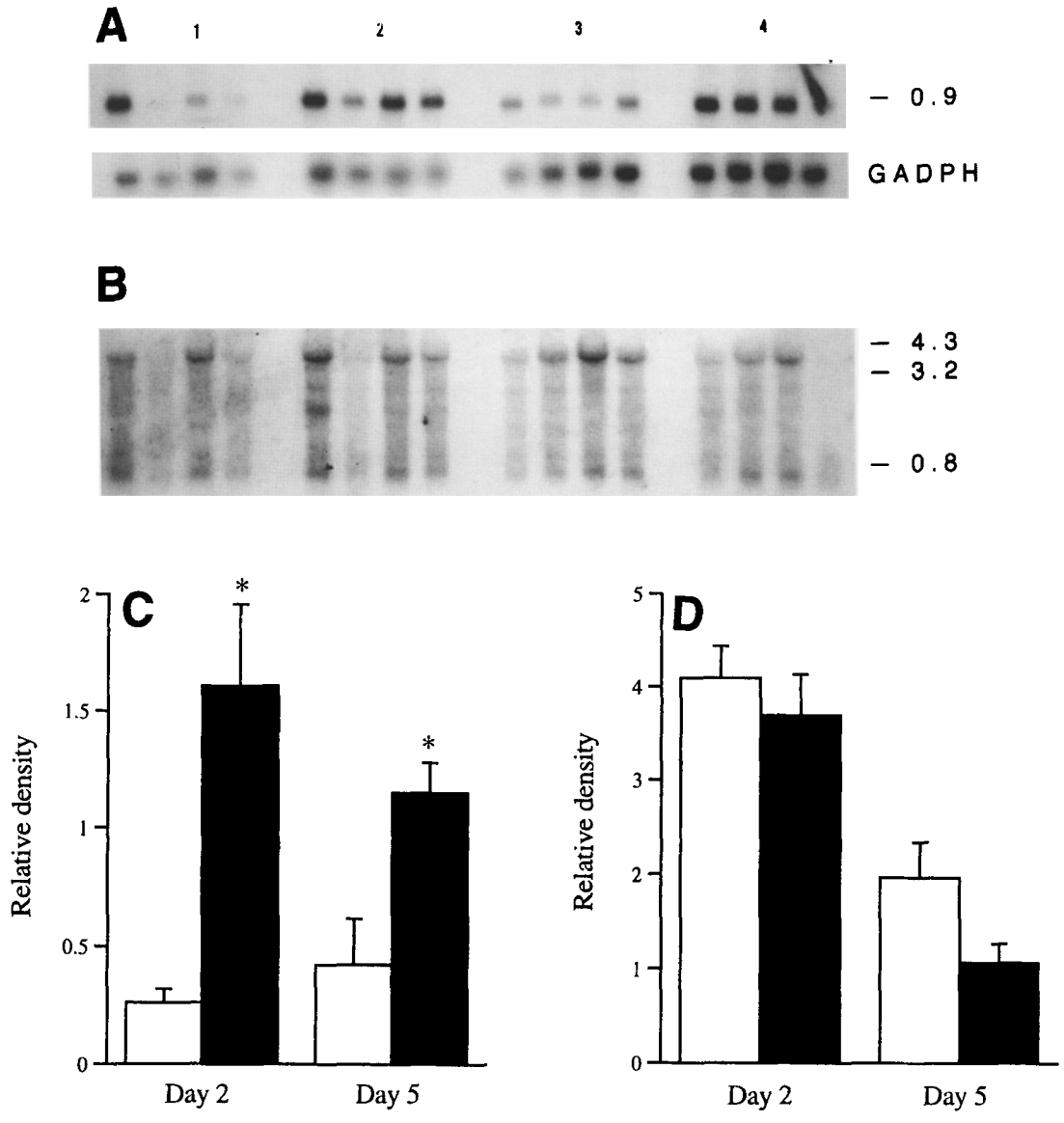

Fig. 3. Northern blot analysis of **(A)** GHRG-I and **(B)** cGHR mRNA in vehicle control or GH-treated adult chickens. Birds were treated with saline (groups 1 and 3) or GH (groups 2 and 4) by osmotic minipumps for either 2 (groups 1 and 2) or 5 (groups 3 and 4) d. Total RNA (15 mg) was prepared, electrophoresed, and transferred to nylon, as described in the Materials and Methods section. The blot was hybridized to cDNA probes for GHRG-I and cGHR. Hybridization of the same blot to GAPDH was used for normalization of loading. Densitometry results for **(C)** GHRG-I and **(D)** GHR. Vehicle (open bars), GH (solid bars). *Vehicle vs GH, within day, $p < 0.05$.

increases hepatic ^{125}I-GH binding, whereas GH replacement therapy reverses this effect *(25)*. Evidence that the increase in hepatic ^{125}I-GH binding sites following hypophysectomy are functional receptors comes from the greater ability of GH to influence monodeiodinase activity in hypophysectomized chicks as compared to intact chicks *(26)*.

Acute GH treatment has been shown to increase plasma concentrations of T_3 in chick embryos, but not young chicks *(27)*. In the adult chicken, acute GH treatment increases circulating concentrations of T_3 in fasted, but not fed, animals *(28)*. Similarly, in the present study using fed adults, GH treatment did not alter plasma concentrations of T_3. However, circulating concentrations of T_4 increased, and consequently the T_3/T_4 ratio decreased. Changes in plasma T_3 are thought to be direct effects of GH on hepatic monodeiodinase(s) *(29)*. The absence of an effect in young chicks may be caused by endogenous GH exerting a maximal effect; administration of antisera to GH acutely reduces circulating concentrations of T_3 *(26)*. Because distinct effects of GH treatment were observed on circulating concentrations of IGF-I and hepatic GHRG-I, it cannot be argued that GH administered as a continuous infusion is ineffective because of GHR downregulation. It is, however, possible that the critical concentration of GH required to influence monodeiodination differs from that needed for other effects. Alternatively, GH may be influencing a number of different points on T_3 and T_4 synthesis, conversion and catabolism such that in the present experiment's paradigm, only minimal effects of GH were found.

No effects of GH treatment were observed on circulating concentrations of NEFAs or glucose. Acute administration of high concentrations of GH to chickens has been previously observed to elevate plasma concentrations of NEFA but to have no effect on plasma glucose *(30)*. Similarly, GH exerts a lipolytic effect on chicken adipose tissue in vitro *(31,32)*. Although no significant change in circulating concentrations of NEFA were observed, GH infusion did reduce the amount of abdominal fat in these chickens. The lack of a hyperglycemic/diabetogenic effect of GH in the chicken is different from some other species, for instance, the pig *(4,33)* and some studies of young chickens *(34)*.

In agreement with mammalian studies, testes weight was not altered by GH treatment. For instance, the administration of GH does not affect testes weights in hypophysectomized *(35)* or dwarf rats with isolated GH deficiency *(36)* and number of Leydig cells *(36)*. It might be noted that GH administration to hypophysectomized rats has been found to increase both the number of macrophage and the cross-sectional area of individual macrophage in the testes *(36)*. The lack of changes in circulating concentrations of LH, follicle stimulating hormone (FSH), or prolactin with GH treatment is in agreement with mammalian studies *(37)*.

Materials and Methods

Animals

The use of animals in this study was approved by the Rutgers University Institutional Review Board for the Use and Care of Animals. Two studies were conducted using adult (>52 wk of age) male white Leghorn chickens. These had been reared (in floor pens) from day-old chicks obtained from Avian Services (Frenchtown, NJ). Immediately prior to experimentation, chickens were transferred to cages and housed individually. Birds were given free access to a commercial layer diet (Agway, Flemington, NJ) and water. On d 0 of each study, chickens were implanted with osmotic minipumps (Model 2 ML, Alzet Corp. Palo Alto, CA) calculated (based on preliminary studies) to deliver 100 μg rc GH/day/kg body weight. Recombinant chicken GH was dissolved in saline containing 0.2% BSA (the rcGH was kindly donated by American Cyanamid, Princeton, NJ). Unimplanted (study 1, n = 6/treatment) chickens or chickens implanted with osmotic pumps containing saline and 0.2% BSA (vehicle, study 2, n = 15/treatment) were used as controls. Blood was collected by venipuncture from the wing vein from all birds on d 2, 5, and 10, following implanting in study 1. Plasma was stored at –20°C until analysis. In study 2, blood was collected similarly from five chickens/treatment on d 2, 5, and 10 then these chickens were killed, organs were weighed, then frozen in liquid nitrogen and stored at –70°C until analysis.

Hormone Assays

Plasma concentrations of GH and IGF-I were determined in both studies by either a homologous (GH) radioimmunoassay (RIA) *(38)* or a heterologous (IGF-I) RIA (*39*; as validated for chicken plasma *see* ref. *40*). The donation of antisera to IGF-I by the National Hormone and Pituitary Program is gratefully acknowledged.

Plasma concentrations of triiodothyronine (T_3) and thyroxine (T_4) were determined using RIA kits (ICN Biomedicals, Irvine, CA). Plasma concentration of FSH *(41)* and plasma LH were determined by RIA kits supplied by the USDA Animal Hormone Program. Plasma prolactin concentrations were also determined by an RIA kit supplied by Al Parlow, Harbor-UCLA Medical Center, Torrance, CA. Samples from each study were assayed in a single RIA, and the intra-assay coefficient of variation was <5%. These RIAs were only performed on plasma from study 2 chickens.

Metabolic Assays

Plasma concentrations of glucose were measured in study 1 (Sigma kit No. 510, St. Louis, MO). Nonesterified fatty acid (NEFA) concentrations in the plasma were determined in study (Wako kit, Wako Chemicals, Dallas, TX).

Ligand-Blotting-IGFBP

Plasma proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), as described by Laemmli *(42)*. Samples were prepared under nonreducing conditions, then boiled for 5 min, and separated on polyacrylamide gels (12%). Proteins were then electrotransferred to polyvinylidene difluoride (PVDF; BioRad, Hercules, CA) membranes.

Following electrotransfer, ligand blotting was performed as described by Hossenlopp et al. *(43)*, with slight modifications. Membranes were dried at 37°C for 5 min, then washed in Tris buffered saline (0.01*M* Tris, TBS) with 3% Nonidet P40 (Sigma) for 30 min and blocked with 1% BSA (Intergen, Purchase, NY) in TBS for 2 h. Following a 10-min wash with TBS plus 0.1% Tween-20 (BioRad, TTBS), membranes were incubated overnight with ^{125}I-human-IGF-I (Amersham, Arlington Heights, IL) in TTBS containing 1% BSA. Excess ligand was removed by multiple TBS and TTBS washes. All incubations and washes were performed at 4°C. Blots were then dried at 37°C, placed in autoradiography cassettes with X-ray film (Hyperfilm MP, Amersham), and developed after 3–5 d at –80°C. Relative changes in IGFBP were assessed by laser densitometry (Pharmacia, Piscataway, NJ).

GH Regulated Gene-I and GH Receptor mRNA

Total RNA was isolated from cells lysed using guanidine thiocyanate *(44)* followed by $CsCl_2$ gradient purification and electrophoresed through an agarose-formaldehyde gel as described previously *(45)*. The RNA was transferred to nylon using a pressure blotter (Posiblotter, Stratagene, La Jolla, CA) and crosslinked with ultraviolet light. The nylon membrane was prehybridized and hybridized to a cDNA probe for GHRG-I as described previously *(13)* and washed at 60°C for 15 min each in 2X SSC 0.1% SDS, 1X

SSC 0.1% SDS, and 0.2X SSC 0.1% SDS (1X = 0.15*M* NaCl, 0.015*M* sodium citrate, pH 7.0). The blot was stripped (100C, 0.2X SSC 0.1% SDS) and reprobed with a probe for the extracellular domain of the chicken GH receptor *(44)*. The blot was then rehybridized with random primer labeled chicken glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA. Quantitation of mRNA was performed using scanning laser densitometry (Molecular Dynamics). Signals for GHRG-I mRNA and cGHR mRNA were normalized to expression of GAPDH.

Statistical Analysis

Data were analyzed by two factor analysis of variance (ANOVA), using time (day) and implant (saline or GH) and their interaction as sources of variation. When interactions were deemed important ($p < 0.20$), means were separated using Bonferroni t-tests *(46)*. Results from densitometry of ligand blots are expressed as a percent of control chickens at each time point. When possible, and no study effect was evident, data were pooled across the two studies. Data from plasma GH, plasma IGF-I, body weights, organ weights, plasma metabolites, and other hormones were analyzed by two factor ANOVA, using time (day) and implant (saline or GH) and their interaction as sources of variation. When interactions were deemed important ($p < 0.20$), means were separated using Bonferroni t-tests *(46)*.

Binding proteins, GHR, and GHRG-I mRNA data were analyzed by one factor ANOVA at each time point. Results from densitometry of ligand blots are expressed as a percent of control chickens at each time point. Data from the analysis of GHRG-I and GHR mRNA are corrected for variations in loading using GAPDH.

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