The Effects of Glucocorticoids (Dexamethasone) on Insulin-Like Growth Factor-I, IGF-Binding Proteins, and Growth in Chickens (44300)

STEVEN LEILI[1] AND COLIN G. SCANES[2]

Department of Animal Sciences, Rutgers—The State University of New Jersey, New Brunswick, New Jersey 08903

Abstract. **The effect of glucocorticoids (dexamethasone) on circulating concentrations of insulin-like growth factor (IGF)-I and IGF binding proteins (IGFBPs) was examined in young (2-week-old) chickens. Increasing dosages (6, 60, and 600 μg/kg) of dexamethasone resulted in progressive growth retardation as evidenced by decreased body-weight gain, reduced bone growth, and breast muscle weights, together with immunosuppression as indicated by decreased thymus and bursa weights. Plasma concentrations of IGF-I were progressively reduced with increased doses of dexamethasone. There were no changes in circulating IGFBPs except on Day 7 when binding activity of the 36-kDa IGFBP was increased by dexamethasone (6 μg/kg). It is speculated that similarities exist between the 36-kDa IGFBP and the mammalian IGFBP-1 based on the response to dexamethasone treatment.** [P.S.E.B.M. 1998, Vol 218]

The present study examined the effects of the glucocorticoid dexamethasone on growth and plasma concentrations of growth hormone (GH) and insulin-like growth factor-I (IGF-I) in young chickens with high or increasing concentrations of GH and IGF-I (1, 2). Reductions in body weight or body-weight gain have been demonstrated in many species including chickens (3–6), humans (7), rats (8–10), and monkeys (11). The mechanisms by which glucocorticoids elicit these effects may include inhibition of GH secretion (12) or impairment of IGF-I production (13, 14). The latter is supported by the ability of exogenous IGF-I to increase growth and circulating concentrations of IGF-I in dexamethasone-treated rats (10). The effects of glucocorticoids on circulating concentrations of IGF-I vary with dose. Decreases in circulating concentrations of IGF-I following glucocorticoid treatment have also been observed in humans (15) and rats (10, 16, 17), and with IGF-I release depressed *in vitro* with rat osteoblast-like cells (18). However, low doses of glucocorticoids increase serum levels of IGF-I in rats (13) and humans (15). While dexamethasone increases circulating concentrations of IGF-I in humans, serum IGF-I bioactivity is decreased, suggesting a shift in insulin-like growth factor binding protein (IGFBP) levels (19).

Few studies have examined the effects of glucocorticoids on IGFBPs in mammals (13, 15, 20) and none in avian species. Six IGFBPs have been identified definitively in mammals. Several roles of IGFBPs have been suggested including serving as a transport mechanism for IGF-I in the blood and modulation of IGF-I tissue specificity (21, 22). Three IGFBPs have been identified in the chicken based on ^{125}I-IGF-I binding (23–27) with an additional IGFBP observed using ^{125}I-cIGF-II as a radioligand (28, 29).

Based on antibody binding, the 30-kDa IGFBP in chickens may be homologous to the human IGFBP-2 (27). The present study examines the effect of glucocorticoids on growth and circulating concentrations of IGF-I and IGFBP in young chicks. It was hypothesized that the expressed growth suppression with glucocorticoids would be paralleled by decreases in plasma concentrations of IGF-I and

[1] Present address and to whom requests for reprints should be addressed at Bristol-Myers Squibb, Pharmaceutical Research Institute, Toxicology Department, Building 125, 1 Squibb Drive, P.O. Box 191, New Brunswick, NJ 08903-0191. E-mail: Steven_.Leili@ccmail.bms.com

[2] Present Address: College of Agriculture, Iowa State University, Ames, IA 50011-1050.

This paper is from the Journal Series, New Jersey Agricultural Experiment Station and the Iowa Agricultural and Home Economic Experiment Station (supported by state and Hatch Act funds).

Received September 8, 1997. [P.S.E.B.M. 1998, Vol 218]
Accepted March 12, 1998.

0037-9727/98/2184-0329$10.50/0

increases in those of IGFBPs just as is observed with nutritional deprivation (30–32).

Materials and Methods

Animals. One-day-old male white Leghorn chicks (Avian Services, Inc., Frenchtown, NJ) were maintained on a commercial chick diet that met NRC recommendations (Country Feeds Broilermaker, soy protein 21% min, Agway, Syracuse, NY) throughout the experiment. After 5 days, chicks were assigned to five treatment groups containing nine chickens per group. Each of the groups was then assigned to one of the five treatments by random assignment. Animals in poor health or with body weights outside an acceptable range (mean ± 2 SDs) were not assigned to the study.

Dexamethasone, in a vehicle of 15% ethanol and 85% phosphate buffered saline (PBS), was administered to three of the five treatment groups at doses of 6, 60, or 600 μg/kg (1 ml/kg of 6, 60, or 600 μg/ml, respectively). Two groups served as controls and received either 85% PBS or the 15% ethanol/85% PBS vehicle at the same dose volume (1 ml/kg) as the dexamethasone treated groups. Since the vehicle contained a large percentage of ethanol, PBS was administered alone in order to differentiate any overt signs of toxicity that may be attributable to the ethanol. Dexamethasone or vehicle was injected subcutaneously on Days 1, 3, and 6 of the experiment. Body weights and shank-toe bone length (as an indicator of skeletal growth) were determined on Days 0 (pretest), 3, and 6. Blood samples were obtained by venipuncture from the brachial vein on Day 0 and following decapitation on Day 7. Breast muscle, bursa, and thymus weights were obtained during necropsies on Day 7.

IGF-I Radioimmunoassay. Plasma concentrations of IGF-I were determined by a heterologous radioimmunoassay (33, as validated for chicken plasma: 34). Plasma concentrations of IGF-I were determined in a single assay to eliminate interassay variance. The coefficient of variation for the IGF-I assay was 3.1%.

IGFBP Analysis. Plasma IGFBPs were identified by and subsequently quantified using radioligand blotting techniques that were slightly modified from those of Hossenlopp *et al.* (35, 36) and previously described in detail by Leili *et al.* (30). The preparation of all plasma samples included an acid extraction prior to analysis in order to dissociate the IGFBPs from the ligand. In addition, the evaluation of nonspecific binding of the IGFBPs had been previously performed in our laboratory [i.e., the addition of nonradioactive IGF-I in excess completely inhibited the binding of the radioactive ligand (36)]. All gels/radiographs contained a control lane that consisted of a pooled sample of 1- and 27-day-old chicken plasma; this standard plasma pool was used for all radioligand assays. The IGFBPs from plasma samples were compared to those found in the standard plasma pool. All mentions of "standard" in the following text concerning binding proteins refers to the pooled plasma "standard." Data are presented as a percentage of the optical density (OD) observed for the standard plasma pool.

Statistical Analysis. Data were analyzed by analysis of variance (ANOVA). Significant differences in means were determined by least significant differences (LSD). Since the results obtained for the two control groups were essentially the same on Day 7, and the body weights and bone lengths were comparable in all groups on Day 0, IGF-I levels and the binding activities of the IGFBPs were assumed to be equally comparable on Day 0 and were accessed on Day 7 only. The IGF-I levels in the control groups were considered baseline, and all data from the dexamethasone-treated groups were compared to those results from the control group receiving the complete vehicle (15% ethanol/85% PBS). Statistical analysis was performed using Excel, Version 5, Analysis ToolPak.

Results

Growth (Body Weight, Shank-Toe Bone Length, and Organ Weights). Control chickens in treatment Groups 1 and 2 (85% PBS and 15% ethanol/85% PBS, respectively) showed steady increases in body weight throughout the experiment. Chicks treated with dexamethasone showed reductions ($P < 0.05$) in body growth compared to the controls (Table I). Average daily body-weight gains were markedly reduced ($P < 0.05$) by dexamethasone

Table I. The Effects of Dexamethasone Treatment on Body Weight and Shank-Toe Bone Length in White Leghorn Chickens

Treatment (dexamethasone μg/kg)	Body weight (g)†			Shank-tone bone length (cm)†		
	Day 0	Day 3	Day 6	Day 0	Day 3	Day 6
0 (85% PBS)	51.1 ± 1.0^{a}	73.3 ± 2.2^{a}	95.2 ± 3.8^{a}	5.5 ± .05^{a}	5.8 ± .06^{a}	6.4 ± .07^{a}
0 (15% Ethanol/85% PBS)	51.4 ± 1.1^{a}	73.2 ± 1.7^{a}	94.9 ± 2.5^{a}	5.4 ± .04^{a}	5.8 ± .04^{a}	6.4 ± .06^{a}
6	51.2 ± 0.9^{a}	71.6 ± 1.7^{a,b}	83.7 ± 2.1^{b}	5.4 ± .05^{a}	5.7 ± .04^{a,b}	6.1 ± .05^{b}
60	51.6 ± 1.0^{a}	68.9 ± 0.9^{a,b}	78.9 ± 0.8^{b,c}	5.4 ± .03^{a}	5.6 ± .04^{b,c}	5.8 ± .05^{c}
600	51.3 ± 1.1^{a}	67.1 ± 1.1^{b}	72.8 ± 1.8^{c}	5.4 ± .03^{a}	5.5 ± .02^{c}	5.6 ± .04^{d}

† Values represent means ± SEM, $n = 9$ chickens/group for all groups.
$^{a-d}$ Means with different superscript letters differ ($P < 0.05$) by Analysis of Variance (ANOVA), followed by Least Significant Differences (LSD).
PBS = Phosphate Buffered Saline.

and PBS vehicle [PBS vehicle 6.3 ± SEM g/day; PBS/ethanol vehicle 6.2 ± SEM g/day; dexamethasone 6 μg, 4.6 ± SEM g/day; 60 μg, 3.9 ± SEM g/day; 600 μg, 3.1 ± SEM g/day]. Reductions in bone growth ($P < 0.05$) were observed in both the 60 and 600-kg dose groups on Day 3 and on Day 6 in all the dexamethasone-treated groups. The control groups showed steady increases in shank-toe bone length during the 7-day observation period. Average shank-toe bone gain was decreased ($P < 0.05$) by dexamethasone [PBS vehicle 0.13 ± SEM mm/day; PBS/ethanol vehicle 0.14 ± SEM mm/day; dexamethasone 6 μg, 0.10 ± SEM mm/day; 60 μg, 0.06 ± SEM mm/day; 600 μg, 0.03 ± SEM mm/day]; the growth was almost completely suppressed (82% reduction) with the highest dexamethasone dose.

Due to the catabolic effects frequently associated with glucocorticoid treatment, breast muscle weights were evaluated (Table II). Mild and marked decreases ($P < 0.05$) in muscle weight were observed in chickens given the 60- and 600-μg/kg dose (17 and 45% lower than controls, respectively). Similar reductions were noted in the weights of the major immune organs (i.e., bursa of Fabricius and thymus), in all the dexamethasone-treated groups (Table II). At doses of 6 and 60 μg/kg, bursa weights were decreased by 27 and 54%, respectively, on Day 7. A substantial decrease (24% of control) was observed in the 600-μg/kg group ($P < 0.05$). Decreases in thymus weights in the 6, 60, and 600 μg/kg groups were 31, 59, and 78% lower than controls on Day 7 ($P < 0.05$).

Circulating Plasma Concentrations of IGF-I. Plasma concentrations of IGF-I in chicks of the control groups (Group 1- 85% PBS and Group 2- 15% ethanol/85% PBS) increased as expected during the 7-day experiment (mean plasma concentrations of 124.2 and 122.7 ng/ml for Groups 1 and 2 on Day 7, respectively, representing a 2 to 3 fold increase from Day 0 of the study). The plasma concentrations of IGF-I were reduced on Day 7 in 60 and 600 μg/kg dexamethasone-treated groups compared to controls (Table III). Plasma concentrations of IGF-I were 28% lower than the controls ($P < 0.05$) in the 60-μg/kg dose group. The most pronounced reduction ($P < 0.05$) in plasma concentrations of IGF-I was observed at a dose of 600 μg/kg (a reduction of 62%).

Circulating Levels of IGFBPs. In this experiment, three IGFBPs binding proteins were characterized using SDS-PAGE electrophoresis. Radiolabeled ^{125}I-human IGF-I bound to specific proteins with MWs of approximately 30, 36, and 40 kDa (Fig. 1). The binding activities of the 30- and 40-kDa IGFBPs were similar to those in the controls on Day 7 following treatment with dexamethasone (Table III). However, the binding activity of the 36-kDa IGFBP was increased ($P < 0.05$) by 64% at 6 μg/kg of dexamethasone on Day 7 (Table III). Binding activity of the 36-kDa IGFBP in the 60- and 600-μg/kg dose groups was comparable to the controls on Day 7.

Discussion

The administration of dexamethasone has been shown to have adverse effects on growth and development (see introduction for references), and this was again observed in the present study (Table I).

The specific mechanism by which glucocorticoids effect growth and growth factors is not well defined. In mammals, the effects of dexamethasone on IGF-I are often dependent upon the dose levels administered. Dexamethasone at low doses has been shown to increase levels of IGF-I in rats (13, 20) and humans (15). No such effect was observed in the low dose of dexamethasone in the present study (Table III). At higher doses, decreases in plasma concentrations of IGF-I have been observed in humans (15) and rats (10, 16). Similarly in the present study with young chickens, reductions in plasma concentrations of IGF-I were observed following the administration of dexamethasone at doses of 60 and 600 μg/kg (Table III). Of particular interest is the lack of tight correlation between the inhibition of growth by dexamethasone and the responses of IGF-I. Marked inhibition of skeletal growth was observed with the lowest dose of dexamethasone (Table I). However, there was not a concomitant reduction on the circulating concentration of IGF-I (Table III).

Despite the reductions in growth and plasma concentrations of IGF-I, there were little changes in the binding activity of IGFBPs. The binding activity of the 30-kDa IGFBP was unaffected by dexamethasone administration. This is in contrast with the increase in this IGFBP with feed

Table II. The Effects of Dexamethasone Treatment on Breast Muscle, Thymus, and Bursa Weights in White Leghorn Chickens

Treatment (dexamethasone μg/kg)	Organ weights (g)† Day 7		
	Breast muscle	Thymus	Bursa
0 (85% PBS)	2.63 ± 0.24^{a}	0.28 ± 0.03^{a}	0.40 ± 0.03^{a}
0 (15% Ethanol/85% PBS)	2.92 ± 0.14^{a,b}	0.32 ± 0.03^{a,b}	0.41 ± 0.03^{a}
6	2.56 ± 0.09^{a,b,c}	0.22 ± 0.01^{a,b,c}	0.30 ± 0.02^{b}
60	2.26 ± 0.07^{a,c}	0.13 ± 0.01^{a,b,c}	0.19 ± 0.02^{c}
600	1.60 ± 0.20^{d}	0.07 ± 0.01^{c}	0.10 ± 0.02^{d}

† Values represent means ± SEM, $n = 9$ chickens/group for all groups.
$^{a-d}$ Means with different superscript letters differ ($P < 0.05$) by Analysis of Variance (ANOVA), followed by Least Significant Differences (LSD).
PBS = Phosphate Buffered Saline.

Table III. The Effects of Dexamethasone Treatment on IGF-I Plasma Concentrations and the Binding Activity of the 30-, 36-, and 40-kDa IGFBPs on Day 7 in White Leghorn Chickens

Treatment (dexamethasone μg/kg)	IGF-I plasma levels (ng/ml)	IGFBP binding activity as optical density† (% of standard)††		
		30-kDa IGFBP	36-kDa IGFBP	40-kDa IGFBP
0 (85% PBS)	124.2 ± 9.8[a]	35.4 ± 11.6[a]	164.9 ± 25.0[a]	806.5 ± 336.0[a]
0 (15% Ethanol/85% PBS)	122.7 ± 11.6[a]	94.2 ± 23.8[a]	139.6 ± 16.5[a]	539.1 ± 278.7[a]
6	92.9 ± 16.1[a,b]	109.6 ± 90.7[a]	229.3 ± 9.9[b]	787.4 ± 254.5[a]
60	88.3 ± 5.5[b]	70.8 ± 15.9[a]	167.6 ± 8.9[a]	785.7 ± 309.7[a]
600	46.9 ± 9.2[c]	63.7 ± 29.5[a]	133.9 ± 15.5[a]	309.6 ± 100.8[a]

† Values represent means ± SEM, $n = 6$ chickens/group for all groups.
†† Plasma obtained and combined from 1- and 27-day-old White Leghorn chickens.
[a–c] Means in columns with different superscript letters differ ($P < 0.05$) by Analysis of Variance (ANOVA), followed by Least Significant Differences (LSD).
PBS = Phosphate Buffered Saline.

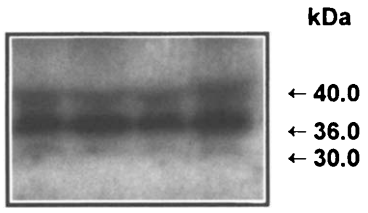

Figure 1. Representative ligand blot of chicken plasma IGF-binding proteins (IGFBP). Plasma proteins were separated by electrophoresis and blotted with ^{125}I-labeled IGF-I as outlined in the text. Proteins with approximate molecular masses of 30, 36, and 40 kDa were identified. Each blot contained a plasma standard in the Lane 1 position (far left lane).

restriction and fasting in young chicks (30). Moreover, feed restriction would be expected to elevate plasma glucocorticoid concentrations. In view of the tentative identification of the 30-kDa chicken IGFBP as IGFBP-2 based on antibody binding (27), it is not surprising that the changes of the 30-kDa IGFBP are not consistent with these of IGFBP-1 in mammals. Suppression of IGFBP-1 has been noted following glucocorticoid treatment in humans (37). In contrast, treatment with low doses of dexamethasone in rats results in increases in IGFBP-1 serum concentrations (32). The only IGFBP affected by dexamethasone in this experiment was the 36-kDa IGFBP (Table III) and that effect was only noted at the lowest dose of dexamethasone administered (6 μg/kg). No changes in binding activity of the 36-kDa IGFBP were noted at 60 and 600 μg/kg. The changes in binding activity of the 36-kDa IGFBP observed in young chicks (Table III) are very similar to those found in rats with IGFBP-1 (32).

In conclusion, as was expected, the glucocorticoid dexamethasone reduced growth in young chickens and induced regression of the immune tissues, thymus, and bursa. Some of the growth/immune suppressive roles of dexamethasone can be attributed to the reductions in the circulating concentrations of IGF-I. It is unlikely that the change in the 36-kDa IGFBP influences growth. Moreover, the failure of dexamethasone to moderate the binding activity of either the 30- or 40-kDa IGFBPs militates against either IGFBP being involved in the growth suppressive effect of dexamethasone. It would therefore appear that glucocorticoids exert their growth/immune suppressive effect both indirectly *via* IGF-I and directly at the tissue level.

We gratefully acknowledge the technical assistance of A. Konopka.

1. Kikuchi K, Buonomo FC, Kajimoto Y, Rotwein P. Expression of insulin-like growth factor-I during chicken development. Endocrinology **128:**1323–1328, 1991.
2. McGuinness MC, Cogburn LA. Measurement of developmental changes in plasma insulin-like growth factor-I levels of broiler chickens by radioreceptor assay and radioimmunoassay. Gen Comp Endocrinol **79:**446–458, 1990.
3. Bartov I, Jensen LS, Veltmann JR Jr. Effect of corticosterone and prolactin on fattening in broiler chicks. Poult Sci **59:**1328–1334, 1980.
4. Nagra CL, Meyer RK. Influence of corticosterone on the metabolism of palmitate and glucose in cockerels. Gen Comp Endocrinol **3:**131–138, 1963.
5. Simpkins JW, Smith GJV. The temporal interaction of corticosterone and prolactin in affecting liver lipid metabolism of the chick. Poult Sci **55:**728–734, 1976.
6. Davison TF, Rea J, Rowell JG. Effects of dietary corticosterone on the growth and metabolism of immature *Gallus domesticus.* Gen Comp Endocrinol **50:**463–468, 1983.
7. Travis RH. Pathophysiology of syndromes of cortisol excess in man. *Handbook of Physiology,* Section 7, Endocrinology, Vol. VI, Adrenal Glands. Washington, DC: American Physiological Society, pp271–282, 1975.
8. Tomas FM, Lemmey AB, Read LC, Ballard FJ. Superior potency of infused IGF-I analogs which bind poorly to IGF-binding proteins is maintained when administered by injection. J Endocrinol **150:**77–84, 1996.
9. Lo HC, Hinton PS, Yang H, Unterman TG, Ney DM. Insulin-like growth factor-I but not growth hormone attenuates dexamethasone-induced catabolism in parenterally fed rats. J Parenter Enteral Nutr **20:**171–177, 1996.
10. Binz K, Schmid C, Bouillon R, Froesch ER, Jurgensen K, Hunziker E. Interactions of insulin-like growth factor I with dexamethasone on trabecular bone density and mineral metabolism in rats. Eur J Endocrinol **130:**387–393, 1994.
11. Marin G, Malozowski SN, Barnes K, Southers J, Cristiano A, Cassoria F. Endocrine profile of catch-up growth in the cynomolgus monkey. Acta Endocrinologica **129:**371–374, 1993.
12. Kaufmann S, Jones KL, Wehrenberg WB, Culler FL. Inhibition of

prednisone of growth hormone (GH) response to GH-releasing hormone in normal man. J Clin Endocrinol Metab **67:**1258, 1988.
13. Luo J, Murphy LJ. Dexamethasone inhibits growth hormone induction of insulin-like growth factor-I (IGF-I) messenger ribonucleic acid (mRNA) in the hypophysectomized rat and reduces IGF-I mRNA abundance in the intact rat. Endocrinology **125:**165–171, 1989.
14. Asakawa K, Takano K, Kogawa M, Hasumi Y, Shizume K. Effects of glucocorticoid on body growth and serum levels of somatomedin A in the rat. Acta Endocrinol **100:**206, 1982.
15. Gourmelen M, Girard F, Binoux M. Serum somatomedin/insulin-like growth factor (IGF) and IGF carrier levels in patients with Cushing's Syndrome or receiving glucocorticoid therapy. J Endocrinol Metab **54:**885–892, 1982.
16. Park JHY, McCusker RH, Mohammadpour H, Blackwood DJ, Hrbek M, Vanderhoof JA. Dexamethasone inhibits mucosal adaptation after small bowel resection. Gastrointest Liver Physiol **29:**G497–G503, 1994.
17. Mosier HD, Jansons RA, Hill RR, Dearden LC. Cartilage sulfation and serum somatomedin in rats during and after cortisone-induced growth arrest. Endocrinology **99:**580–589, 1976.
18. Chen TL, Mallory JB, Hintz RL. Dexamethasone and 1,25(OH)2 vitamin D3 modulate the synthesis of insulin-like growth factor-I in osteoblast-like cells. Calcif Tissue Int **48:**278–282, 1991.
19. Miell JP, Taylor AM, Jones J, Holly JMP, Gaillard RC, Pralong FP, Ross RJM, Blum WF. The effects of dexamethasone treatment on immunoreactive and bioactive insulin-like growth factors (IGFs) and IGF-binding proteins in normal male volunteers. J Endocrinol **136:**525–533, 1993.
20. Binoux M, Lassarre C, Hardouin N. Somatomedin production by rat liver organ culture. III. Studies on the release of insulin-like growth factor and its carrier protein measured by radioligand assays: Effects of growth hormone, insulin, and cortisol. Acta Endocrinol **99:**422–430, 1982.
21. Hintz RL. Plasma forms of somatomedin and the binding protein phenomenon. Clin Endocrinol Metab **13:**31–42, 1984.
22. Radetti G, Paganini C, Antoniazzi F, Pasquino B, Valentini R, Gentili L, Tato L. Growth hormone-binding proteins, IGF-I and IGF-binding proteins in children and adolescents with type 1 diabetes mellitus. Horm Res **47:**110–115, 1997.
23. Armstrong DG, McKay CO, Morrell DJ, Goddard C. Insulin-like growth factor-I binding proteins in serum from the domestic fowl. J Endocrinol **120:**373–378, 1989.
24. Francis GO, McMurtry JP, Johnson RJ, Ballard F. Plasma clearance of chicken and human insulin-like growth factor-I and their association with circulating binding proteins in chickens. J Endocrinol **124:**361–370, 1990.
25. Lee PDK, Peacock A, Roessler MK, Hester J, Reeves JT. Insulin-like growth factor (IGF)-I and IGF-binding activity in normal and fast-growing chickens. Life Sci **45:**2465–2470, 1989.
26. Lord APD, Bastian SEP, Read LC, Walton PE, Ballard F. Differences in the association of insulin-like growth factor-I (IGF-I) and IGF-I variants with rat, sheep, pig, human, and chicken plasma binding proteins. J Endocrinol **140:**475–482, 1994.
27. Morishita D, Sasaki K, Wakita M, Hoshino S. Effect of fasting on serum insulin-like growth factor-I (IGF-I) levels and IGF-I-binding activity in cockerels. J Endocrinol **139:**363–370, 1993.
28. McMurtry JP, Francis GO, Upton Z, Walton PE, Rosselot G, Caperna TJ, Brocht DM. Plasma clearance and tissue distribution of labeled chicken and human IGF-I and IGF-II in the chicken. J Endocrinol **150:**149–160, 1996.
29. Schoen T, Beebe D, Clemmons D, Chader G, Waldbillig R. Local synthesis and developmental regulation of avian vitreal insulin-like growth factor-binding proteins: A model for independent regulation in extravascular and vascular compartments. Endocrinology **131:**2846–2854, 1992.
30. Leili S, Buonomo FC, Scanes CG. The effects of dietary restriction on insulin-like growth factor-I, -II, and IGF-binding proteins in chickens. Proc Soc Exp Biol Med **216:**104–111, 1997.
31. Leili S, Scanes CG. The effects of protein restriction on insulin-like growth factor-I and IGF-binding proteins in chickens. Proc Soc Exp Biol Med **218:**322–328, 1998.
32. Luo J, Reid RE, Murphy LJ. Dexamethasone increases hepatic insulin-like growth factor binding protein-1 (IGFBP-1) mRNA and serum IGFBP-1 concentrations in the rat. Endocrinology **127:**1456–1462, 1990.
33. Furlanetto RW, Underwood LE, VanWyk SS, D'Ercole A. Estimation of somatomedin-C levels in normal patients with pituitary disease by radioimmunoassay. J Clin Invest **60:**648–657, 1977.
34. Huybrechts LM, King DB, Lauterio TJ, Marsh J, Scanes CG. Plasma concentrations of somatomedin-C in hypophysectomized, dwarf, and intact growing domestic fowl as determined by heterologous radioimmunoassay. J Endocrinol **104:**233–239, 1985.
35. Hossenlopp P, Seurin D, Segovia-Quinson B, Hardonin S, Binoux M. Analysis of serum insulin-like growth factor binding proteins using Western Blotting: Use of the method for titrating of the binding proteins and competitive binding studies. Anal Biochem **154:**138–143, 1986.
36. Radecki SV, Capdevielle MC, Buonomo FC, Scanes CG. Ontogeny of insulin-like growth factors (IGF-I and IGF-II) and IGF-binding proteins in the chicken following hatching. Gen Comp Endocrinol **107:**109–117, 1997.
37. Miell JP, Taylor AM, Zini M, Maheshwari H, Ross RJM, Valcavi R. Effects of hypothyroidism and hyperthyroidism on insulin-like growth factors (IGFs) and IGF and growth hormone (GH) binding proteins. J Clin Endocrinol Metab **76:**950–955, 1993.