PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 185, 420–426 (1987)

# Time Course of Changes in Plasma Concentrations of the Growth Related Hormones during Protein Restriction in the Domestic Fowl (*Gallus domesticus*) (42563)

THOMAS J. LAUTERIO* AND COLIN G. SCANES†

*Washington University School of Medicine, Department of Internal Medicine, Metabolism Division, St. Louis, Missouri 63110, and †Department of Animal Sciences, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08903*

*Abstract.* Experiments were conducted to evaluate the possible role of circulating growth hormones triiodothyronine ($T_3$), thyroxine ($T_4$), and insulin-like growth factor I (somatomedin-C; IGF-I) in the elevation of plasma growth hormone (GH) which occurs in protein-restricted chickens. Plasma hormone changes were determined over a 2-week period of protein depletion by feeding a 5% protein diet as well as a similar period of protein repletion with a 20% protein diet. The rise in plasma GH was observed in two separate studies. Plasma concentrations of $T_4$, $T_3$, and IGF-I were all depressed in protein-restricted chicks prior to or concurrent with the GH elevation. In the protein repletion time course study, $T_4$ and $T_3$ concentrations were normalized prior to or concurrent with plasma GH normalization. However, IGF-I concentrations in repleted chicks did not return to control levels until after normal levels of GH were observed. These data suggest that thyroid hormones may play a greater role in the regulation of GH secretion during periods of malnourishment than IGF-I; the latter being currently thought to be a peripherally circulating inhibitor of GH release in animals. 

Chickens fed a low protein diet for 2 weeks have an increased plasma concentration of growth hormone (GH) when compared with that of adequately fed controls (1). A similar phenomenon is seen in humans (2, 3), but not in rats which have decreased plasma GH concentrations in response to protein restriction (4, 5). Although it has been shown that rats have an accompanying decrease in pituitary GH content with protein deficiency (4), no mechanism has been established for GH changes observed in the chicken. Obviously the events leading to the changes are as different as the end results when comparing the two species.

Other hormonal changes also occur during protein or protein–calorie restriction. For example, there is a decrease in plasma concentrations of insulin-like growth factor I (somatomedin-C; IGF-I) in the chicken (6), rat (7, 8), and human (9, 10). Thyroid function and weight decrease under these conditions in the chicken (11), and plasma concentrations of thyroid hormones decrease in the human (3) but are unaffected (12) or increase (13) in the rat. Thus, the human metabolic responses to protein restriction are more similar to those of the chicken than to those of the rat.

It is possible that the increases in the plasma concentrations of GH reflect elevated secretion due to a decrease in negative feedback from IGF-I, triiodothyronine ($T_3$), or thyroxine ($T_4$). Indeed IGF-I has been observed to exert a negative feedback inhibition of GH release *in vitro* (14) and *in vivo* (15, 16). Although thyroid hormones are not major inhibitors of GH release in mammals, there is an inverse relationship between plasma thyroid hormones and GH secretion in chickens. For instance, chicks having reduced thyroid function due to goitrogen administration (17) or autoimmune thyroiditis (18) have elevated plasma GH concentrations. Sex-linked dwarf chickens with inherent depressed plasma $T_3$ concentrations also have greater plasma concentrations of GH than the normals of the parent strain (19). Daily injections of $T_3$ and $T_4$ reduce circulating GH concentrations in young chickens (20). Thus it is possible that the plasma GH elevation seen with protein restriction may be due, at least in part, to the depression of these proposed negative feedback inhibitors ($T_3$, $T_4$, and IGF-I) of GH secretion. For these hormones to be further implicated in this metabolic response, a change in the circulating levels must occur to or simultaneous with GH changes. In a series of time course studies, these possibilities were explored.

**Materials and Methods.** Male White Leghorn day-old chicks (*Gallus domesticus*) were



0037-9727/87 $1.50

obtained from Avian Services (Frenchtown, N.J.) and reared in Petersime brooder batteries throughout the entire study. Room temperature was controlled at 22 ± 1°C while photoperiod was maintained at 16L:8D. Birds were fed chick starter diet (Agway Corp., New Brunswick, NJ) until experimental diets were fed and water was always available. Ingredients used to mix purified experimental 5 and 20% protein diets were purchased from Dyets, Inc. (Bethlehem, PA). Composition of these isocaloric diets have been described elsewhere (21). Food consumption and growth data for birds maintained on these diets are also described in this previous report.

*Experiment 1.* Chicks received the purified 20% protein diet from age 2 to 4 weeks to acclimate birds to the texture and palatability changes of the purified diets compared to the commercial feed. At 4 weeks of age birds were distributed into treatment groups having equivalent body weights ($N = 7$) and assigned either the control 20% protein or the low 5% protein diet. Diets were isocaloric with the difference in energy being supplied as carbohydrate (cornstarch). Texture and palatability of these diets were equivalent as determined by food consumption data of previous experiments (21). An initial (time = 0 days) group was sacrificed at that point by decapitation and blood was collected. Plasma was then obtained by centrifugation and stored at −20°C for hormone analysis. Birds were then sacrificed in the same manner as above 1, 2, 3, 4, 5, 6, 8, 10, 12 and 14 days following the start of the diets.

*Experiment 2.* In this trial, chicks were fed either the 5 or 20% protein diets from age 2 to 4 weeks. At that point, one-half of the birds in each treatment group were switched to the alternate diet, while the remainder continued their original feeding regime. At Time 0 and Days 1, 2, 3, 4, 6, 8, 10, 12 and 14 after the change in diets, chicks were sacrificed as above and blood was collected for hormone analysis.

*Hormone analysis.* GH concentrations were measured using the homologous radioimmunoassay of Harvey and Scanes (22). Plasma concentrations of IGF-I were assayed by the heterologous RIA method of Furlanetto *et al.* (23) validated for the chicken in our laboratory (24). The antisera and the iodinated IGF-I were gifts of Drs. J. J. Van Wyk and Louis E. Underwood with the former being obtained through the National Hormone and Pituitary Program. Standards for this assay were dilutions of pooled plasma from 6- to 8-week-old chickens; 1 unit being equivalent to the amount of IGF-I in 1 ml of a laboratory standard pool of chicken plasma. Both plasma $T_4$ and $T_3$ were assayed using the component kits from Antibodies, Inc. (Davis, CA) and the iodinated hormone was purchased from Amersham Radiochemicals (Arlington Heights, IL).

*Statistics.* Hormone concentration differences between low protein and control birds in the first time course study were compared using Student's *t* test for unpaired observations (25). For the second time course study, data were treated by analysis of variance and the means were separated by the Duncan multiple range test.

**Results.** Figures 1 and 2, respectively, illustrate plasma concentrations of GH and IGF-I (Fig. 1) and those of $T_3$ and $T_4$ (Fig. 2) after initiation of feeding a low protein diet. Plasma concentrations of GH in the protein-restricted chicks were significantly elevated ($P < 0.05$) compared with control chicks on Days 8, 12, and 14 of the experimental diet. On the other hand, plasma concentrations of IGF-I were decreased ($P < 0.001$) in the birds fed low protein by Day 2 of the dietary regime and remained lower ($P < 0.05$) than controls

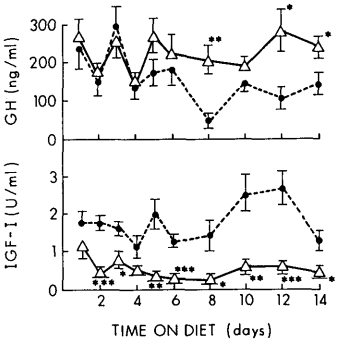


FIG. 1. Plasma concentrations of GH and IGF-I in cockerels fed 5 or 20% protein diets from 4 to 6 weeks of age. Birds were preacclimated to 20% protein (control) purified diets for 2 weeks prior to start of time course. Data from chicks fed 5% protein diets are represented by open triangles, whereas data from chicks receiving the 20% protein (control) diet are represented by closed circles. Asterisks indicate values different from controls on the same day. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

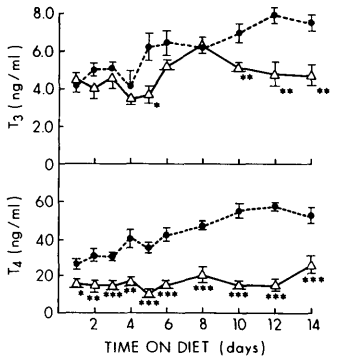


FIG. 2. Plasma concentrations of $T_3$ and $T_4$ in cockerels fed 5 or 20% protein synthetic diets from 4 to 6 weeks of age. Birds were preacclimated to 20% protein (control) purified diets for 2 weeks prior to start of the time course. Data from chicks fed 5% protein diets are represented by open triangles whereas data from chicks receiving the 20% protein diet are represented by closed circles. Asterisks indicate values different from controls on the same day. $^{*}P < 0.05$, $^{**}P < 0.001$, $^{***}P < 0.001$.

throughout the rest of the study. The mean difference between dietary groups varied greatly over the 2-week period with controls averaging 3.6-fold greater concentrations than malnourished. Plasma concentrations of IGF-I were 10-fold greater in controls on Days 10 and 12 than their protein-restricted counterparts. Plasma concentrations of $T_3$ and $T_4$ were also affected in the protein-restricted birds (Fig. 2). Chickens fed 5% protein had decreased plasma concentrations of $T_3$ on Days 5, 10, 12, and 14 compared to controls. The plasma concentrations of $T_4$ in the low protein group were decreased 1 day after being fed experimental diets with the mean plasma concentration of $T_4$ being reduced by 60% from Day 1 to 14 compared to controls.

The second study involved both protein restriction and repletion of 2- to 6-week-old chicks. During the initial 2-week period chicks were fed either 5 or 20% protein diets from age 2 to 4 weeks. At that point, one-half of each initial group continued to receive the same diet while the other half was switched to the alternate diet. Data on plasma concentrations of GH, IGF-I, $T_3$, and $T_4$ are presented in Tables I through IV. Plasma concentrations of GH after 2 weeks of being fed the low protein diet were consistent with the previous study; being three-fold higher than those of controls. However, by Day 1 of protein repletion, plasma GH concentrations were reduced 38.5% compared with chicks remaining on the 5% protein diet. By Day 4 of repletion, plasma concentrations of GH did not differ from chicks fed the 20% protein diet throughout the study. Birds switched from 20 to 5% protein showed the characteristic rise in GH concentrations by Day 8 compared to controls and these levels remained higher through Day 14. Birds fed 5% protein diets for the entire ex-

TABLE I. EFFECT OF A LOW PROTEIN DIET AND PROTEIN REPLETION ON PLASMA CONCENTRATIONS OF GH IN CHICKS

| Days after start of second feeding | Protein level fed (age 2–4 weeks/age 4–6 weeks) | | | |
|---|---|---|---|---|
| | Group 1 5%/5% | Group 2 5%/20% | Group 3 20%/20% | Group 4 20%/5% |
| 0 | 456.0 ± 74.9 (8)$^{b}$ | | 152.4 ± 16.8 (8)$^{a}$ | |
| 1 | 423.9 ± 43.2 (8)$^{c}$ | 260.4 ± 54.3 (8)$^{b}$ | 149.8 ± 25.8 (7)$^{a}$ | 179.5 ± 22.3 (8)$^{a,b}$ |
| 2 | 336.2 ± 27.1 (8)$^{b}$ | 288.8 ± 59.1 (8)$^{b}$ | 182.9 ± 33.1 (8)$^{a}$ | 145.5 ± 10.9 (8)$^{a}$ |
| 3 | 603.9 ± 50.6 (7)$^{c}$ | 294.6 ± 37.8 (8)$^{b}$ | 183.4 ± 34.4 (8)$^{a}$ | 260.2 ± 31.0 (8)$^{a,b}$ |
| 4 | 316.2 ± 27.1 (8)$^{c}$ | 227.9 ± 22.3 (8)$^{a}$ | 157.9 ± 26.4 (8)$^{a}$ | 227.7 ± 28.9 (8)$^{a}$ |
| 6 | 401.6 ± 60.3 (7)$^{b}$ | 137.0 ± 24.4 (8)$^{a}$ | 146.4 ± 27.0 (6)$^{a}$ | 223.0 ± 32.9 (8)$^{a}$ |
| 8 | 342.8 ± 53.3 (8)$^{c}$ | 170.9 ± 30.2 (8)$^{b}$ | 88.1 ± 9.6 (8)$^{a}$ | 230.9 ± 28.6 (8)$^{b}$ |
| 10 | 476.9 ± 184.0 (6)$^{b}$ | 201.0 ± 27.3 (7)$^{a}$ | 156.9 ± 24.1 (8)$^{a}$ | 310.3 ± 46.7 (8)$^{b}$ |
| 12 | 369.9 ± 70.9 (7)$^{b}$ | 91.3 ± 14.8 (8)$^{a}$ | 176.8 ± 23.4 (8)$^{a}$ | 348.2 ± 72.7 (7)$^{b}$ |
| 14 | 331.0 ± 29.3 (8)$^{b}$ | 83.5 ± 14.4 (8)$^{a}$ | 98.3 ± 23.1 (8)$^{a}$ | 313.8 ± 38.4 (7)$^{b}$ |

*Note.* Birds were fed 5 and/or 20% protein diets from 2 to 4 and 4 to 6 weeks of age. Data are presented as means (ng/ml) ± standard error ($N$).

$^{a,b,c}$ Means with the same letter are not different ($P < 0.05$ or less) on same day by ANOVA and Duncans multiple range test.

TABLE II. EFFECT OF A LOW PROTEIN DIET AND PROTEIN REPLETION ON PLASMA CONCENTRATIONS OF IGF-I IN CHICKS

| Days after start of second feeding | Protein level fed (age 2–4 weeks/age 4–6 weeks) | | | |
|---|---|---|---|---|
| | Group 1 5%/5% | Group 2 5%/20% | Group 3 20%/20% | Group 4 20%/5% |
| 0 | 2.13 ± 0.14 (8)[a] | | 3.38 ± 0.39 (8)[a] | |
| 1 | 2.29 ± 0.15 (8)[a] | 2.27 ± 0.17 (8)[a] | 3.11 ± 0.15 (8)[b] | 2.90 ± 0.15 (8)[b] |
| 2 | 2.07 ± 0.09 (8)[a] | 1.75 ± 0.15 (7)[a] | 3.53 ± 0.37 (8)[b] | 2.05 ± 0.29 (8)[a] |
| 3 | 1.95 ± 0.10 (8)[a,b] | 2.20 ± 0.13 (8)[b] | 2.86 ± 0.17 (8)[b,c] | 1.55 ± 0.11 (8)[a] |
| 4 | 1.29 ± 0.12 (8)[a,b] | 1.78 ± 0.16 (8)[b] | 3.34 ± 0.15 (8)[c] | 1.27 ± 0.09 (8)[a] |
| 6 | 1.14 ± 0.09 (6)[a] | 2.16 ± 0.34 (8)[b] | 3.01 ± 0.25 (8)[c] | 1.78 ± 0.17 (8)[b] |
| 8 | 1.33 ± 0.13 (7)[a] | 2.16 ± 0.31 (8)[b] | 2.84 ± 0.36 (8)[b] | 1.28 ± 0.17 (b)[a] |
| 10 | 1.41 ± 0.17 (6)[a] | 3.13 ± 0.32 (7)[b] | 3.25 ± 0.47 (8)[b] | 1.54 ± 0.13 (7)[a] |
| 12 | 1.62 ± 0.16 (7)[a] | 2.99 ± 0.54 (8)[b] | 3.53 ± 0.20 (8)[b] | 1.77 ± 0.23 (7)[a] |
| 14 | 1.28 ± 0.12 (8)[a] | 3.57 ± 0.49 (8)[b] | 3.34 ± 0.15 (8)[b] | 1.21 ± 0.12 (8)[a] |

*Note.* Birds were fed 5 and/or 20% protein diets from 2 to 4 and 4 to 6 weeks of age. Data are presented as means (U/ml) ± standard error (*N*).

[a,b,c] Means with the same letter are not different ($P < 0.05$ or less) on same day by ANOVA and Duncans multiple range test.

perimental period had elevated plasma concentrations of GH compared to controls at all time points.

Plasma concentrations of IGF-I in protein-restricted chickens were 40% lower than controls after 2 weeks of diet consumption. Protein depletion during the second feeding period further decreased plasma concentrations of IGF-I. Chicks fed the low protein diet in the second half of the study had decreased plasma concentrations of IGF-I by Day 2 of protein depletion and these concentrations remained lower through the course of the experiment. Plasma concentrations of IGF-I increased during protein repletion such that levels in this group were similar to those in protein-repleted chicks on Days 3, 8, 10, 12, and 14 of the study.

Plasma concentrations of $T_3$ were 32% lower in protein-malnourished chicks compared to controls at 4 weeks of age (Table 3). Birds switched from 5 to 20% protein had in-

TABLE III. EFFECT OF A LOW PROTEIN DIET AND PROTEIN REPLETION ON PLASMA CONCENTRATIONS OF $T_3$

| Days after start of second feeding | Protein level fed (age 2–4 weeks/age 4–6 weeks) | | | |
|---|---|---|---|---|
| | Group 1 5%/5% | Group 2 5%/20% | Group 3 20%/20% | Group 4 20%/5% |
| 0 | 5.2 ± 0.1 (8)[a] | | 7.6 ± 0.7 (8)[b] | |
| 1 | 3.8 ± 1.0 (7)[a] | 10.1 ± 0.5 (7)[b,c] | 11.3 ± 1.5 (8)[c] | 7.6 ± 1.0 (8)[b] |
| 2 | 4.4 ± 0.5 (6)[a] | 11.5 ± 1.4 (7)[c] | 12.0 ± 0.6 (7)[c] | 8.4 ± 0.6 (6)[b] |
| 3 | 4.2 ± 0.4 (7)[a] | 16.4 ± 1.2 (7)[c] | 10.9 ± 0.7 (7)[b] | 6.4 ± 1.1 (7)[a] |
| 4 | 6.8 ± 1.2 (6)[a] | 14.9 ± 1.6 (8)[c] | 11.8 ± 0.7 (8)[b,c] | 8.4 ± 1.2 (6)[b] |
| 6 | 3.6 ± 0.7 (6)[a] | 13.0 ± 0.8 (7)[c] | 11.9 ± 1.3 (8)[b] | 6.7 ± 1.8 (7)[a] |
| 8 | 4.9 ± 0.5 (8)[a] | 14.4 ± 1.1 (8)[b] | 12.4 ± 1.6 (8)[b] | 7.5 ± 1.8 (8)[a] |
| 10 | 5.1 ± 0.9 (6)[a] | 14.6 ± 1.2 (7)[c] | 15.6 ± 0.8 (6)[c] | 9.2 ± 2.0 (7)[b] |
| 12 | 6.7 ± 0.7 (6)[a] | 10.8 ± 1.4 (7)[b] | 13.5 ± 2.0 (8)[b] | 5.3 ± 0.9 (8)[a] |
| 14 | 4.1 ± 1.1 (7)[a] | 14.8 ± 2.1 (8)[b] | 13.2 ± 0.8 (7)[b] | 4.8 ± 1.1 (7)[a] |

*Note.* Birds were fed 5 and/or 20% protein diets from 2 to 4 and 4 to 6 weeks of age. Data are presented as means (ng/ml) ± standard error (*N*).

[a,b,c] Means with the same letter are not different ($P < 0.005$ or less) on same day by ANOVA and Duncans multiple range test.

creased plasma concentrations of $T_3$ by one day of repletion compared to protein-restricted chicks. Plasma concentrations of $T_3$ were equal to or greater (Day 4) than protein-repleted controls until Day 14. Switching the diet from 20 to 5% protein caused a decrease in plasma concentrations of $T_3$ similar to that of the previous study. This effect was consistant after 6 days of receiving the diet. Chicks fed 5% protein diet throughout the entire study and those switched from 20 to 5% protein had consistently lower plasma concentrations of $T_4$. Birds receiving the low protein diet for the second two-week feeding period required 10 days of food consumption to achieve the low levels observed in birds fed at the 5% level for both periods. Within 3 days of switching chicks from 5 to 20% protein, plasma concentrations of $T_4$ were increased compared to restricted birds.

**Discussion.** The GH response to protein restriction was examined in two time course studies of a 2- and 4-week duration, respectively, and the possible role of the thyroid hormones and/or IGF-I in this response was evaluated. It has been suggested in several reports that IGF-I acts as a negative feedback inhibitor of GH secretion (Berelowitz *et al.* (14) and Tannenbaum (15)). The former study demonstrated a depression in GH release *in vitro* by addition of purified IGF-I to rat pituitary cell incubations. The latter report implicated IGF-I as an *in vivo* inhibitor of GH secretion. A semipurified preparation rich in multiplication-stimulating activity (MSA) and somatomedin-C (IGF-II and IGF-I, respectively) injected into the lateral ventricle, inhibited pulsatile GH release 2 hr after administration. It is possible, therefore, that a decrease in IGF-I due to protein restriction results in a reduction of negative feedback of GH secretion. Thyroid hormones have also been shown to affect plasma GH concentrations in the chicken (17–20).

Results from the first time course study (Figs. 1 and 2) indicate that when plasma concentrations of GH in protein-restricted chicks were elevated consistently by 12 and 14 days after receiving the diet, plasma concentrations of $T_3$, $T_4$, and IGF-I were depressed compared with those of controls. In the second time course study, plasma concentrations of GH in the protein-repleted chicks returned to normal 4 days after being switched to the 20% protein diet. Plasma concentrations of $T_3$ in this repleted group recovered within 1 day, and plasma concentrations of $T_4$ returned to control levels by Day 4. The fact that plasma $T_4$ concentrations recover from protein restriction later than plasma $T_3$ concentrations may indicate that conversion of $T_4$ to $T_3$ rather than total thyroid hormone synthesis is primarily affected. Recent studies have indicated that factors influencing circulating thyroid hormone concentrations can independently effect liver 5′-monodeiodinase activity and the thyroid gland (26, 27). It is possible, therefore, that effects of protein restriction on thyroid hormone concentrations could occur at the liver and alter the circulating ratio of $T_3$ to $T_4$ without changes in plasma concentrations. Plasma IGF-I concentrations in repleted chicks, however, were still lower than those of controls 4 and 6 days after receiving the 20% protein diet. Results of these two studies suggest that although IGF-I may be a negative feedback inhibitor of GH secretion, a clear relationship between IGF-I and GH concentrations is not evident. The repletion time course study (Tables I-IV) demonstrates normal concentrations of GH can exist with low-plasma concentrations of IGF-I. On the other hand, these studies make a strong case for the involvement of the thyroid hormones. Plasma concentrations of both $T_3$ and $T_4$ are depressed in protein-restricted chicks prior to GH increases. However, plasma concentrations of $T_3$ returned to normal almost immediately after protein repletion while changes in the plasma concentrations of GH and $T_4$ coincided. A possible mechanism for this interrelationship may involve thyrotropin-releasing hormone (TRH) which stimulates both GH- and thyroid-stimulating hormone (TSH) release from the pituitary (28, 29). Growth hormone is also secreted in response to growth hormone-releasing factor (GRF) (30). It could follow that low levels of thyroid hormones, induced by protein deficiency, stimulate TRH release from the hypothalamus. Another possibility is that $T_3$ and/or $T_4$ exert direct inhibitory effects on the pituitary gland decreasing both basal and secretagogue-induced release of GH and TSH. The mechanism underlying the reduction in plasma concentrations of IGF-I in protein deficiency is unknown at this point. Syn-

TABLE IV. EFFECT OF A LOW PROTEIN DIET AND PROTEIN REPLETION ON PLASMA CONCENTRATIONS OF $T_4$

| Days after start of second feeding | Protein level fed (age 2–4 weeks/age 4–6 weeks) | | | |
|---|---|---|---|---|
| | Group 1 5%/5% | Group 2 5%/20% | Group 3 20%/20% | Group 4 20%/5% |
| 0 | 12.9 ± 0.7 (8)[a] | | 44.4 ± 2.3 (8)[b] | |
| 1 | 11.6 ± 0.8 (8)[a] | 13.6 ± 1.0 (6)[a] | 30.1 ± 1.1 (8)[c] | 22.2 ± 1.9 (8)[b] |
| 2 | 12.5 ± 1.9 (8)[a] | 15.7 ± 1.6 (8)[a] | 36.4 ± 2.3 (8)[c] | 27.3 ± 2.2 (8)[b] |
| 3 | 10.4 ± 0.5 (7)[a] | 26.7 ± 2.4 (7)[b] | 35.7 ± 3.8 (8)[c] | 19.2 ± 2.5 (7)[b] |
| 4 | 11.5 ± 1.0 (8)[a] | 34.3 ± 1.9 (8)[c] | 32.2 ± 1.8 (8)[c] | 20.1 ± 1.7 (8)[b] |
| 6 | 12.2 ± 1.2 (7)[a] | 26.0 ± 1.7 (8)[c] | 29.7 ± 1.3 (8)[c] | 20.3 ± 1.6 (8)[b] |
| 8 | 14.0 ± 1.0 (8)[a] | 34.3 ± 0.9 (7)[c] | 39.2 ± 2.2 (8)[d] | 22.6 ± 2.0 (8)[b] |
| 10 | 15.0 ± 1.4 (6)[a] | 34.5 ± 1.3 (7)[b] | 38.8 ± 2.5 (8)[b] | 18.5 ± 1.3 (8)[a] |
| 12 | 9.4 ± 1.4 (6)[a] | 36.7 ± 3.5 (8)[b] | 34.1 ± 1.6 (8)[b] | 11.2 ± 2.1 (6)[a] |
| 14 | 11.7 ± 1.8 (6)[a] | 32.4 ± 3.4 (8)[b] | 32.2 ± 1.7 (7)[b] | 14.7 ± 1.1 (8)[a] |

*Note.* Birds were fed 5 and/or 20% protein diets from 2 to 4 and 4 to 6 weeks of age. Data are presented as means (ng/ml) ± standard error (*N*).

[a–d] Means with the same letter are not different ($P < 0.005$ or less) on same day by ANOVA and Duncans multiple range test.

thesis of IGF-I may also require the presence of thyroid hormones at some level, or the alteration of nutritional substrates may exert direct effects on IGF synthesis and/or release. Although it is well documented that plasma concentrations of IGF-I decrease with protein deficiency in a variety of species (2, 6–9), the role of $T_3$ or $T_4$ in this response has not been investigated. Lower circulating levels of IGF-I could also aid in the protein-restricted increase in plasma concentrations of GH as IGF-I has been shown to inhibit GH secretion (14, 15). It is possible that initial decreases in plasma concentrations of IGF-I could aid in elevating plasma GH concentrations. Increased circulating levels of GH would in turn down-regulate GH receptors at the periphery and further depress IGF-I synthesis and release. As this cycle continued, an amplification of the problem would occur until plasma GH concentrations leveled off and plasma IGF-I concentrations bottomed out.

These speculations require investigation. The complexity of the thyroid–pituitary–hypothalamic axis has made such studies difficult as the system is both dynamic and multidirectional. Further studies are underway to elucidate the role of thyroid hormones in the dietary-induced increase in GH concentrations.

Paper of Journal Series, New Jersey Agricultural Experiment Station, Project 18141, supported by State and Hatch Act funded and a grant from the National Science Foundation (PCM-8302197). The authors wish to acknowledge and thank Ms. Phyllis Anderson for preparation of this manuscript.

1. Scanes CG, Griminger P, Buonomo FC. Effects of dietary protein restriction on circulating concentrations of growth hormone in growing domestic fowl (*Gallus domesticus*). Proc Soc Exp Biol Med **168**:334–337, 1981.
2. Hintz RL, Suskind R, Amatayakul K, Thanangkul O, Olson R. Plasma somatomedin and growth hormone values in children wtih protein-calorie nutrition. J Pediatrics **92**:153–156, 1978.
3. Munro WN, Crim MC. The proteins and amino acids. In: Goodhart RS, Shils ME, eds. Modern Nutrition in Health and Disease. Philadelphia: Lea and Febiger, Vol. IV, p51–112, 1980.
4. Srebnik HH, Nelson MM, Simpson ME. Reduced growth hormone content in anterior pituitaries of rats on protein-free diets. Proc Soc Exp Biol Med **101**:97–99, 1959.
5. Schalch DS, Mauer KL, Schalch KN, Miller LL. Growth retardation secondary to malnutrition and hypothyroidism: Is there a common defect in the growth promoting system? Proceedings of the 62nd Annual Meeting of the Endocrine Society. Endocrinol **107**:149, 1980. [Abstract]
6. Lauterio TJ, Huybrechts L, Griminger P, Scanes CG. The effect of dietary protein on circulating concentrations of growth hormone, somatomedin-C and protein turnover in the domestic fowl. Fed Proc **43**: 465, 1984. [Abstract]
7. Schalch DS, Burstein PJ, Tewel SJ, Draznin B, Emler CA. The effect of renal impairment on growth in the rat: Relationship to malnutrition and serum somato-

medin levels. Endocrinology **108**:1683–1689, 1981.
8. Prewitt TE, D'Ercole AJ, Switzer BR, Van Wyk JJ. Relationship of serum immunoreactive somatomedin-C to dietary protein and energy in growing rats. J Nutr **112**:144–150, 1982.
9. Phillips LS, Vassilopoulou-Sellin R. Nutritional regulation of somatomedin. Amer J Clin Nutr **32**:1082–1096, 1979.
10. Isley WL, Underwood LE, Clemmons DR. Dietary components that regulate serum somatomedin-C levels in humans. J Clin Invest **71**:175–182, 1983.
11. March BE, Biely J, Pastro KR. The effect of protein level and amino acid balance upon thyroid activity in the chick. Canad J Biochem **42**:341–344, 1964.
12. Glass AR, Mellitt R, Burman KD, Wartofsky L, Swerdloff RS. Serum triiodothyronine in undernourished rats: Dependence on dietary composition rather than total calorie or protein intake. Endocrinology **102**:1925–1928, 1978.
13. Tulp OL, Krupp PP, Danforth E Jr, Horton ES. Characteristics of thyroid function in experimental protein malnutrition. J Nutr **109**:1321–1332, 1979.
14. Berelowitz M, Szabo M, Frohman LA, Firestone S, Chu L, Hintz RL. Somatomedin-C mediates growth hormone negative feedback by effects on both the hypothalamus and the pituitary. Science **212**:1279–1281, 1981.
15. Tannenbaum GS, Guyda HJ, Posner BI. Negative feedback and body weight regulation via brain. Science **113**:1319–1324, 1983.
16. Abe T, Molitch ME, Van Wyk JJ, Underwood LE. Human growth hormone and somatomedin-C suppress the spontaneous release of growth hormone in unanesthetized rats. Endocrinology **113**:1319–1324, 1983.
17. Chiasson RB, Sharp PJ, Klandorf H, Scanes CG. Effect of rapeseed meal and methimazole on levels of plasma hormones in growing broiler cockerels. Poult Sci **58**: 1575–1583, 1979.
18. Scanes CG, Gales L, Harvey S, Chadwick A, Newcomer WS. Endocrine studies in young chickens of the obese strain. Endocrinology **30**:419–423, 1976.
19. Scanes CG, Marsh J, Decuypere E, Rudas P. Abnormalities in the plasma concentrations of thyroxine, triiodothyronine and growth hormone in sex-linked and autosomal dwarf White Leghorn domestic fowl (*Gallus domesticus*). J Endocrinol **97**:127–135, 1983.
20. Harvey S. Thyroid hormones inhibit growth hormone secretion in domestic fowl. (*Gallus domesticus*). J Endocrinol **96**:329–334, 1983.
21. Lauterio TJ, Scanes CG. Hormonal responses to protein restriction in two strains of chickens with different growth characteristics. J Nutr **117**:758–763, 1987.
22. Harvey S, Scanes CG. Purification and radioimmunoassay of chicken growth hormone. J Endocrinol **73**:321–330, 1977.
23. Furlanetto RW, Underwood LE, Van Wyk JJ, D'Ercole AJ. Estimation of somatomedin-C levels in normals and patients with pituitary disease by radioimmunoassay. J Clin Invest **60**:648–657, 1977.
24. Huybrechts LM, King DB, Lauterio TJ, Marsh J, Scanes CG. Plasma concentrations of somatomedin-C in hypophysectomized dwarf and intact growing domestic fowl by heterologous radioimmunoassay. J Endocrinol **104**:233–239, 1984.
25. Steel RGD, Torrie JH. Principles and Procedures of Statistics. New York, McGraw-Hill, 1960.
26. Borges M, Labourene J, Ingbar SH. Changes in hepatic iodothyronine metabolism during ontogeny of the chick embryo. Endocrinology **107**:1751–1761, 1980.
27. Decuypere E, Scanes CG, Kuhn ER. Effects of glucocorticoids on circulating concentrations of thyroxine ($T_4$) and triiodothyronine ($T_3$) and on peripheral monodeiodination in pre- and post-hatching chickens. Horm Metab Res **15**:233–236, 1983.
28. Harvey S, Scanes CG, Chadwick A, Bolton NJ. The effect of thyrotropin-releasing hormone (TRH) and somatostatin (GHRIH) on growth hormone and prolactin secretion *in vitro* and *in vivo* in the domestic fowl (*Gallus domesticus*). Neuroendocrinology **26**: 249–260, 1978.
29. Scanes CG, Harvey S, Bolaffi J. TRH stimulation of *in vivo* GH release in the domestic fowl. Neuroendocrinology **40**:33–38, 1985.
30. Scanes CG, Carsia RV, Lauterio TJ, Huybrechts L, Rivier J, Vale W. Synthetic human pancreatic growth hormone releasing factor (hpGRF) stimulates growth hormone secretion in the domestic fowl (*Gallus domesticus*). Life Sci **32**:1127–1134, 1984.

---

Received November 26, 1986. P.S.E.B.M. 1987, Vol. 185.
Accepted April 16, 1987.