The Effects of Dietary Restriction on Insulin-Like Growth Factor (IGF)–I and II, and IGF-Binding Proteins in Chickens

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Abstract. **The effect of feed (energy/protein) restriction on circulating concentrations of insulin-like growth factor (IGF)–I and II, and IGF-binding proteins (IGFBPs) was examined in young (4-week-old) chickens. Increasing levels of feed restriction caused progressive growth retardation, as evidenced by decreased body-weight gain and reduced bone growth. Plasma concentrations of both IGF-I and IGF-II were decreased, and the degree of reduction in the plasma concentrations of these growth factors appeared to be related to the magnitude of feed restriction. A tendency for greater decreases in these growth factors appeared to be associated with greater feed restriction at the majority of time points evaluated. However, nutritional restriction had a greater effect on plasma concentrations of IGF-I than on those of IGF-II. The reductions in plasma concentrations of IGF-I were observed earlier in the experiment and at a lower degree of nutritional deprivation than for plasma concentrations of IGF-II, possibly suggesting greater sensitivity of IGF-I plasma concentrations to feed restriction. Three IGFBPs with molecular weights of 30, 36, and 40 kDa were detected by radioligand assay following separation by SDS-electrophoresis. The 30-kDa IGFBP was most affected by feed restriction with binding activity of this IGFBP increased by 2 days of feed restriction irrespective of the degree of feed deprivation. The binding activity of the 36-kDa IGFBP was increased, albeit transiently, on the second day of feed restriction. Nutritional restriction had no discernible effect on the binding activity of the 40-kDa IGFBP. Increases in the binding activity of the 30-kDa IGFBP appeared to correspond with the observed decreases in IGF-I plasma concentrations. This suggests decreased bioavailability of IGF-I, and possibly IGF-II, attributed to the formation of a complex between IGF-I and the 30-kDa IGFBP during feed restriction. The initial increase in binding activity of the 36-kDa IGFBP may suggest that this binding protein also plays a role in the regulation and availability of circulating concentrations of IGF-I and IGF-II. Although the binding activity of the 40-kDa IGFBP was unaffected by feed restriction, we can not exclude its importance in the regulation of IGF-I. The substantial binding activity of the 40-kDa IGFBP observed in this experiment suggests that it is one of the major chicken IGFBPs, and that its role in IGF-I regulation warrants further study.** [P.S.E.B.M. 1997, Vol 216]

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Circulating concentrations of insulin-like growth factor–I (IGF-I) have been shown to be influenced by nutritional quality and quantity. Fasting results in a decrease in plasma concentrations of IGF-I in a variety of species, including humans (1), pigs (2), and chickens (3). Although a decrease in dietary caloric intake causes a reduction in plasma concentrations of IGF-I, this decrease can be prevented in humans through the administration of a diet high in protein (4). Refeeding has also been shown to increase plasma concentrations of IGF-I to near normal status following fasting in humans (5). Similarly, dietary protein restriction decreases IGF-I plasma concentrations in hu-

mans (6) and in protein-restricted chickens (7, 8). Conversely, administration of high-fat and high-carbohydrate diets also results in a reduction in plasma concentrations of IGF-I in humans (4). In many species, including humans, sheep, cattle, dogs, and chickens, nutritional energy restriction results in an increase in circulating concentrations of growth hormone (GH) due to the loss of negative feedback from IGF-I (9–13). In meat-type chickens, fasting for a duration of 4 or 7 days has been shown to have no effect on IGF-II levels (14). The present study examines the effects of graduations of feed restriction (from a 20% decrease) to complete feed deprivation on plasma concentrations of IGF-I and IGF-II in young chickens.

In mammals, six IGF-binding proteins (IGFBPs) have been definitively identified. Three IGFBPs have been identified in chickens; these have similar associations to IGF-I as they do in mammals (15–19). In chickens, an additional IGFBP with a mass of approximately 70 kDa has been identified when using 125I–cIGF-II as a radioligand (20, 21). Fasting can affect the plasma levels and/or binding activity of IGFBPs in mammals (rats [22–25], sheep [26], guinea pigs [27, 28]) and chickens (19). Similarly, fasting induces an increase in IGFBP-1 and 2 mRNAs in collagen in guinea pigs (27). There is relatively little information on the changes in binding activity of IGFBPs with physiological state in birds. However, fasting for 5 days elevated the binding activity of the 30-kDa IGFBP (19). The 30-kDa IGFBP in chickens may be homologous to the human IGFBP-2 (19). This 30-kDa IGFBP also behaves similarly to the human IGFBP-2 following hypophysectomy (19). However, circulating levels of IGFBP-1 in mammals have also been shown to increase during fasting (5).

The present study examines the effects of progressive feed (energy/protein) restriction on plasma concentrations of IGF-I and IGF-II, and on the binding activity of IGFBPs in young growing chickens. This is to attempt to define the relationship between energy/protein availability and components of the IGF system.

Materials and Methods

Animals. One-day-old white Leghorn male chicks (Avian Services, Inc., Frenchtown, NJ) were maintained on a commercial feed (Country Feeds Broilermaker, crude protein 21% minimum, crude fat 3% minimum, and crude fiber 4% maximum; Agway, Syracuse, NY, 13221) until 2 weeks old. At this time, chicks were assigned to six treatment groups. Each of the groups were then assigned randomly to one of the six treatments. Animals in poor health or with body weights outside an acceptable range (mean ± 2 SD) were not assigned to the study.

The six groups of chicks (n = 9/group) were provided with feed at the following amounts relative to control *ad libitum* intake: 0%, 20%, 40%, 60%, 80%, and 100%. Body weights, shank-toe bone length (as an indicator of skeletal growth), and food consumption were determined daily. Blood samples were obtained by venipuncture from the brachial vein after 2, 4, or 8 days on study or following decapitation on Day 16. Plasma samples were maintained at −20°C until analysis. Feed restriction was accomplished by means of a staggered feeding system (by 1 day) throughout the study. Average daily feed intake per chicken in the control *ad libitum* group ranged from 7.0 to 47.8 g (mean: 24.6) per day. The amount of feed allocated to each of the feed-restricted treatment groups was dependent solely on the intake of the control *ad libitum* group and was calculated on a daily basis. The treatment groups that received 0%, 20%, and 40% of the control diet amount were sacrificed on Days 2, 4, and 8 of the study, respectively.

IGF-I and IGF-II Radioimmunoassay. Plasma concentrations of IGF-I were determined by a heterologous radioimmunoassay (29; as validated for chicken plasma: Ref. 30). Plasma concentrations of IGF-II were also determined by radioimmunoassay as described by Buonomo *et al.* (31). Plasma concentrations of IGF-I and IGF-II were determined in duplicate in single assays in order to eliminate interassay variance. The intraassay coefficient of variation for the IGF-I and IGF-II assays were 7.4% and 10.4%, respectively.

IGFBP Analysis. Plasma IGFBPs were identified by and subsequently quantified using radioligand blotting techniques slightly modified from those of Hossenlopp *et al.* (32). Molecular sizes of the isolated IGFBPs were estimated using prestained sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) low-range standards (Bio-Rad Laboratories, Hercules, CA). Briefly, this entailed the following. Plasma samples (10 μl of plasma combined with 25 μl of SDS sample buffer [see below]) were boiled for 5 min. Proteins were separated by SDS discontinuous slab PAGE (Mini-PROTEAN II Ready Gels, 4% stacking and 12% separating acrylamide gels; Bio-Rad Laboratories) under nonreducing conditions. The sample buffer contained 10% glycerol, 12.5% 0.5 *M* Tris-HCl, pH 6.8, 2% SDS, and 0.00125% bromophenol blue. Following electrophoresis, the separated proteins were electrotransferred to polyvinylidene difluoride membranes (Bio-Rad Laboratories) for 1 hr at 100 V using a transfer buffer containing 20% methanol, 25 m*M* Tris, and 192 m*M* glycine, pH 8.3. Following electrotransfer, the membranes were dried at 37°C then washed for 30 min in a ligand blot buffer containing 0.15 *M* NaCl, 0.01 *M* Tris, pH 7.4, 0.5 mg/ml sodium azide, and 3% Nonidet P-40 (Sigma Chemical Co., St. Louis, MO). Membranes were then blocked with 1% bovine serum albumin (BSA) (Intergen, Purchase, NY) in Tris-buffered saline (TBS) for 2 hr followed by a 10-min wash with TBS plus 0.1% Tween 20 (TTBS, Bio-Rad Laboratories). The nitrocellulose transfer membranes were incubated overnight with [^{125}I]human IGF-I (300,000 cpm/ml; Amersham, Arlington Heights, IL) in TTBS containing 1% BSA. Subsequently, membranes were washed multiple times with TBS and TTBS to remove unbound ligand. All incubations were performed at 4°C. Blots were then dried at 37°C and placed in a film cassette containing intensifying screens and x-ray

film (Hyperfilm MP, Amersham). The films were exposed for 3–7 days at −80°C. Radiographs were analyzed using laser densitometry (Pharmacia, Piscataway, NJ) to quantify the approximate relative changes in IGFBPs. All radiographs contained a control lane, which consisted of a pooled sample of 1- and 27-day-old chicken plasma (the same standard plasma pool was used for all radioligand assays). The IGFBPs from plasma samples were compared with those found in the standard plasma pool. All mentions of "standard" in the following text concerning binding proteins are referring to the pooled plasma "standard." Data are presented as a percentage of the optical density (O.D.) 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). Statistical analysis performed using Excel, Version 5, Analysis ToolPak.

Results

Growth (Body Weight, Shank-Toe Bone Length) and Food Consumption. As might be expected, chickens in the control group (receiving 100% feed intake) exhibited steady increases in body weight throughout the experiment. All feed-restricted groups showed reduced ($P < 0.05$) body weight compared with the *ad libitum* fed controls (Table I); these being manifest for the 80% intake group by Day 8 of treatment and for ≤60% intake groups by Day 2 of treatment. It might be noted chicks receiving feed intake equivalent to 40% *ad libitum* controls maintained body weight throughout the study. None of the treatment groups at either 2 or 4 days of treatment showed any difference in bone growth (Table II). As expected, the control group showed a steady increase in shank-toe bone length during the 16-day observation period. However, decreases ($P < 0.05$) in shank-toe bone length were noted by Day 8 in the chicks subjected to 40% or 60% feed restrictions and by Day 16 in those chicks receiving a 20% reduction in feed intake. Chickens in both the 40% and 60% feed intake groups showed a 5.8% decrease in shank-toe length by Day 8, progressing to a 10.7% decrease on Day 16. Those animals receiving 80% of control diet had a 6.8% decrease by Day 16.

Circulating Plasma Concentrations of IGF-I and IGF-II. Aside from a slight decrease observed on Day 4, plasma concentrations of IGF-I in chicks of the control 100% *ad libitum* group increased ($P < 0.05$) between Day 4 and Day 8 of the experiment. In the feed-restricted groups, plasma concentrations of IGF-I were decreased ($P < 0.05$) by Day 2 of feed restriction (Fig. 1A). Plasma concentrations of IGF-I in the feed-restricted groups (feed intake: 0%, 20%, 40%, 60%, and 80% of *ad libitum* controls) were 22%, 17%, 26%, 29%, and 49% of those in the control 100% group, respectively. However, these reductions in plasma concentrations of IGF-I were not noted on Day 4, except in the group receiving 20% of the control diet amount ($P < 0.05$). At Day 8, plasma concentrations of IGF-I were reduced ($P < 0.05$) for the 40%, 60%, and 80% feed-restricted groups by 18%, 26%, and 37% of the *ad libitum* fed controls, respectively. Plasma concentrations of IGF-I in the 60% and 80% feed-restricted groups were reduced similarly to approximately half the plasma level of the control group on Day 16.

Plasma concentrations of IGF-II were also decreased by feed restriction. However, the reductions in plasma concentrations of IGF-II were delayed and of a smaller magnitude (Fig. 1B) compared with decreases in IGF-I. For instance, there were no differences in plasma concentrations of IGF-II with level of feeding on Day 2. By Day 4 of treatment, the chickens receiving 40% of control diet had decreased ($P < 0.05$) plasma concentrations of IGF-II, the reduction being 60% compared with 82% with IGF-I. Plasma concentrations of IGF-II in the 60% and 80% groups were not decreased until Days 8 and 16 of feed restriction, respectively (Fig. 1B).

Binding Activity of IGFBPs. In this experiment, three IGFBPs were characterized using SDS-PAGE electrophoresis. Radiolabeled [^{125}I]human IGF-I bound to specific proteins with molecular weights of approximately 30, 36, and 40 kDa (Fig. 2).

Table I. The Effects of Feed Restriction on Body Weight in White Leghorn Chickens

Treatment* (%)	Body weight† (g)				
	0 days‡	2 days	4 days	8 days	16 days
100	122.9 ± 4.4^a	140.6 ± 5.0^a	148.8 ± 6.7^a	196.0 ± 9.0^a	322.0 ± 11.1^a
80	131.5 ± 4.6^a	135.2 ± 3.6^a	153.2 ± 4.3^a	173.6 ± 4.2^b	260.5 ± 7.0^b
60	129.4 ± 3.6^a	112.8 ± 4.9^b	130.2 ± 5.6^b	145.0 ± 4.3^c	210.7 ± 3.4^c
40	126.8 ± 3.1^a	119.8 ± 3.0^b	126.1 ± 2.9^b	128.0 ± 3.0^d	ND
20	125.9 ± 5.4^a	110.3 ± 4.3^{b,c}	108.3 ± 3.5^c	ND	
0	127.2 ± 4.1^a	107.7 ± 3.4^c	ND		

* amount of feed, percentage of control diet administered.
† Values represent the mean ± SEM; $N = 9$ chickens/group for all groups. ND, not determined.
‡ Duration of treatment.
$^{a-c}$ Means in a column with different superscript letters differ ($P < 0.05$) by analysis of variance (ANOVA), followed by least significant differences (LSD).

Table II. The Effects of Feed Restriction on Shank-Toe Bone Length in White Leghorn Chickens

Treatment* %	Shank-toe bone length† (cm)				
	0 days‡	2 days	4 days	8 days	16 days
100	7.2 ± 0.07[a]	7.7 ± 0.08[a]	7.9 ± 0.09[a]	8.6 ± 0.15[a]	10.3 ± 0.14[a]
80	7.4 ± 0.10[a]	7.7 ± 0.13[a]	7.9 ± 0.12[a]	8.4 ± 0.10[a]	9.6 ± 0.10[b]
60	7.4 ± 0.09[a]	7.6 ± 0.10[a]	7.7 ± 0.11[a]	8.1 ± 0.12[b]	9.2 ± 0.07[c]
40	7.5 ± 0.07[b]	7.7 ± 0.08[a]	7.9 ± 0.06[a]	8.1 ± 0.04[b]	ND
20	7.5 ± 0.08[b]	7.7 ± 0.09[a]	7.8 ± 0.07[a]	ND	
0	7.5 ± 0.09[b]	7.7 ± 0.06[a]	ND		

* amount of feed, percentage of control diet administered.
† Values represent the mean ± SEM, $n = 9$ chickens/group for all groups. ND, not determined.
‡ Duration of treatment.
[a–c] Means in a column with different superscript letters differ ($P < 0.05$) by ANOVA, followed by least significant differences (LSD).

Table III summarizes the binding activity of the 30-kDa IGFBP in chicks on *ad libitum* and restricted feeding. Nutritional restriction was accompanied by an increase in the binding activity of the 30-kDa IGFBP, albeit not significantly in some instances. For example, after 4 days of feed restriction the binding activity of the 30-kDa IGFBP was elevated ($P < 0.05$) in the chicks in the 80% treatment group. On Day 8, the 30-kDa IGFBPs were observed to be increased ($P < 0.05$) in the 40% feed-restricted group.

The most abundant IGFBP was that with a molecular weight of approximately 36 kDa (Fig. 2). This binding protein was observed in all plasma samples. Feed restriction for neither 4 nor 8 days affected the binding activity of the 36-kDa IGFBP (Table IV). On Day 2, the binding activity of the 36-kDa IGFBP was increased ($P < 0.05$) in the 0% and 40% feeding groups. However, by Day 16 the binding activity of the 36-kDa IGFBP was decreased ($P < 0.05$) in the chicks receiving 40% the intake of the *ad libitum* fed controls. The second most prominent IGFBP was that with a molecular weight of approximately 40 kDa (Fig. 2). This binding protein was observed in all plasma samples. No significant differences in the binding activity of the 40-kDa IGFBP were noted among the feed-restricted groups at any day studied.

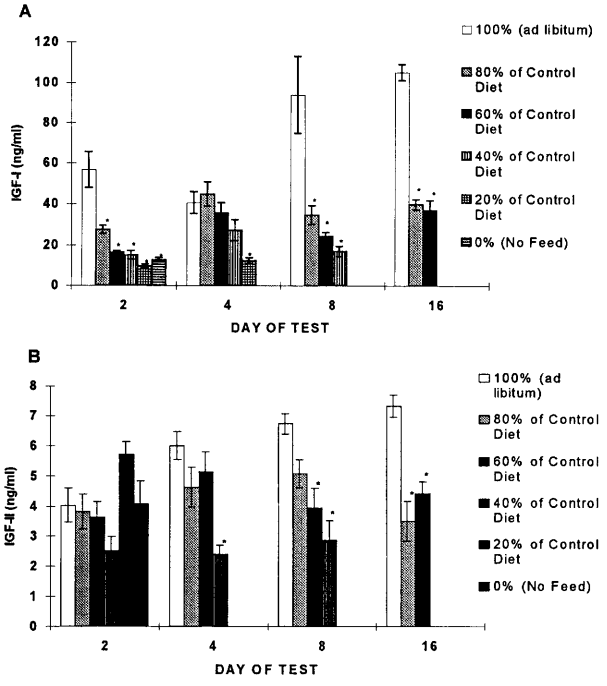

Figure 1. The effects of feed restriction on plasma concentrations of (A) IGF-I and (B) IGF-II in chickens receiving 80%, 60%, 40%, 20%, and 0% of *ad libitum* (100%)–fed controls. The 0%, 20%, and 40% groups were sacrificed on Days 2, 4, and 8, respectively. The 60%, 80%, and 100% groups were sacrificed on Day 16. IGF-II data were not determined for the 20% group on Day 4 due to insufficient sample. The values are mean ($n = 9$) ± SEM. *$P < 0.05$.

Discussion

Young cockerels at a rapidly growing age were chosen for this experiment since effects on growth might be expected to be more evident during this critical stage of rapid growth and development. Even very modest feed restriction (e.g., reductions in intake of 20%) resulted in decreases in body and skeletal growth (Tables I and II). Similarly, plasma concentrations of IGF-I in all feed-restricted groups were reduced (Fig. 1A). It should be noted that even mild feed restriction (80% group) resulted in a marked decline in plasma concentrations of IGF-I. This finding is in agreement with reported reductions in IGF-I concentrations caused by starvation or feed restriction in other species such as rats (22–24, 33, 34), cattle (35), pigs (2), red deer (36), and humans (1, 37, 38). In chickens, a decrease in plasma concentrations of IGF-I is noted following feed restriction (3, 19). Dietary protein restriction has also been shown to decrease plasma concentrations of IGF-I in chickens (7). In a more recent study with chicks, a marked reduction in circulating plasma concentrations of IGF-I was also noted

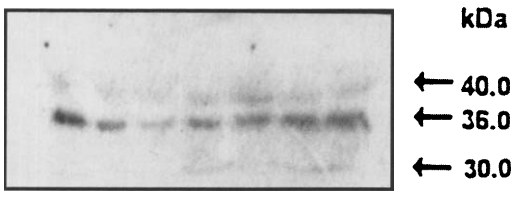

Figure 2. Representative ligand blot of chicken plasma IGFBP. Plasma proteins were separated by electrophoresis and blotted with [^{125}I]-labeled IGF-I as outlined in the text. Proteins with molecular masses of approximately 30, 36, and 40 kDa were identified.

Table III. The Effects of Feed Restriction on the 30-kDa IGFBP in White Leghorn Chickens

Treatment* (%)	30-kDa IGFBP as optical density† (% of standard)‡			
	2 days§	4 days	8 days	16 days
100	53.8 ± 13.4[a]	29.9 ± 9.2[a]	22.3 ± 7.2[a]	172.8 ± 83.7[a]
80	269.6 ± 126.5[a]	77.2 ± 22.7[b]	46.0 ± 12.7[a]	212.8 ± 59.2[a]
60	235.0 ± 91.3[a]	35.0 ± 14.3[a]	69.7 ± 25.5[a,b]	411.2 ± 278.1[a]
40	103.2 ± 14.1[a]	50.1 ± 10.8[a,b]	120.6 ± 31.3[b]	ND
20	127.5 ± 39.7[a]	39.7 ± 2.8[a,b]	ND	
0	140.0 ± 46.5[a]	ND		

* amount of feed, percentage of control diet administered.
† Values represent the mean ± SEM, $N = 6$ chickens/group for all groups. ND, not determined.
‡ Plasma obtained and combined from 1- and 27-day-old white Leghorn chickens.
§ Duration of treatment.
[a–b] Means in a column with different superscript letters differ ($P < 0.05$) by ANOVA, followed by least significant differences (LSD).

during prolonged starvation, with maximal reductions observed after 5 days (19). Plasma concentrations of IGF-I were restored following refeeding, although still lower than controls (19). Reduced circulating concentrations of IGF-I induced by fasting in chickens are correlated with increased GH synthesis (3) and release (9). Since increased GH secretion during feed restriction appears to have little or no effect in preventing either skeletal or body growth retardation in young chickens (the GH–IGF-I axis being uncoupled), growth may be more dependent upon normal IGF-I levels (30, 39). In the present study, growth retardation (body weight and skeletal) appeared to be directly related to the plasma concentrations of IGF-I.

Plasma concentrations of IGF-II were reduced in nearly all the feed-restricted groups by Day 8 of the experiment (Fig. 1B). This finding is consistent with those found during nutritional deprivation in rats (40), humans (41, 42), and swine (31). However, compared with IGF-I concentrations these effects appeared to require a longer duration of feed restriction in order to become evident, especially in those groups with smaller degrees of feed restriction. It should be noted that in rats (43) and meat-type chickens (14) short-term fasting has little or no effect on plasma concentrations of IGF-II and that chronic nutritional deprivation in rats is normally required to elicit this response (40). It appears that the same criteria for feed deprivation as required in rats (i.e., a longer duration of deprivation) may be needed in order to elicit a change in IGF-II plasma concentrations in chickens. Although slight decreases in plasma concentrations of IGF-II were evident on Day 4 (40% group), reductions were not noted until later in the 60% and 80% groups compared with that with IGF-I. It is likely that the mechanism leading to the decline in plasma concentrations of IGF-I during feed restriction (whether it is GH independent or by the loss of GH receptors [i.e., uncoupling of the GH–IGF-I axis]) differs from that evoking the decrease in IGF-II. On the basis of the present study, IGF-I is affected more quickly and to a greater extent then IGF-II by feed restriction. Thus, although IGF-II may contribute to post-hatch growth in the chick, it probably is less important than IGF-I.

In the present study, three IGFBPs, with molecular weights of approximately 30, 36, and 40 kDa, were observed using Western ligand blotting techniques. Similar proteins have been found both in layer-type (19), broiler-type (15), and meat-type chickens (14) and in an IGF-I competitive binding study in chickens (18). Changes observed in the binding activity of the 30-kDa IGFBP agree with previously reported data in which the 30-kDa IGFBP appeared to be sensitive to various physiological changes such as hyperthyroidism, hypophysectomy (44), and fasting (19). The increase in the 30-kDa binding protein with feed restriction in the present study was accompanied by a de-

Table IV. The Effects of Feed Restriction on the 36-kDa IGFBP in White Leghorn Chickens

Treatment* (%)	36-kDa IGFBP as optical density† (% of standard)‡			
	2 days§	4 days	8 days	16 days
100	29.2 ± 5.5[a]	30.3 ± 6.1[a]	21.8 ± 3.0[a]	35.5 ± 7.1[a]
80	45.6 ± 8.0[a,b]	28.2 ± 5.3[a]	26.7 ± 2.4[a]	24.4 ± 3.0[a,b]
60	41.2 ± 8.7[a,b]	27.2 ± 4.0[a]	19.1 ± 3.6[a]	21.2 ± 0.8[b]
40	54.5 ± 10.2[b]	41.5 ± 6.7[a]	23.6 ± 5.8[a]	ND
20	51.6 ± 9.1[a,b]	32.5 ± 3.4[a]	ND	
0	55.6 ± 10.1[b]	ND		

* amount of feed, percentage of control diet administered.
† Values represent the mean ± SEM, $n = 6$ chickens/group for all groups. ND, not determined.
‡ Plasma obtained and combined from 1- and 27-day-old white Leghorn chickens.
§ Duration of treatment.
[a–b] Means in a column with different superscript letters differ ($P < 0.05$) by ANOVA, followed by least significant differences (LSD).

Table V. The Effects of Feed Restriction on the 40-kDa IGFBP in White Leghorn Chickens

Treatment* (%)	40-kDa IGFBP as optical density† (% of standard)‡			
	2 days§	4 days	8 days	16 days
100	58.9 ± 15.9[a]	52.0 ± 8.1[a]	58.8 ± 8.7[a]	56.6 ± 9.8[a]
80	69.4 ± 17.2[a]	37.3 ± 5.8[a]	55.1 ± 5.4[a]	55.0 ± 11.1[a]
60	79.3 ± 18.9[a]	38.2 ± 8.1[a]	39.7 ± 11.3[a]	61.4 ± 6.8[a]
40	84.2 ± 19.3[a]	43.5 ± 8.6[a]	50.7 ± 12.6[a]	ND
20	75.7 ± 17.3[a]	37.9 ± 6.3[a]	ND	
0	77.9 ± 8.6[a]	ND		

* amount of feed, percentage of control diet administered.
† Values represent the means ± SEM, $n = 6$ chickens/group for all groups. ND, not determined.
‡ Plasma obtained and combined from 1- and 27-day-old white Leghorn chickens.
§ Duration of treatment.
[a] Means in columns with different superscript letters differ ($P < 0.05$) by ANOVA, followed by least significant differences (LSD).

crease in plasma concentrations of IGF-I and IGF-II. The increased binding activity of this protein would obviously be expected to influence the availability of the already reduced circulating concentration of IGF-I and IGF-II. It may be argued that there might be homology between this 30-kDa IGFBP and the IGFBP-1 found in mammals since a marked increase in IGFBP-1 is also noted during fasting (5, 45). However, its sensitivity to physiological changes such as hypophysectomy might suggest homology to mammalian IGFBP-2 or -3 (44).

Binding activity of the 36-kDa IGFBP was also influenced by feed restriction (Table IV). However, two opposite effects on this IGFBP were observed (an initial increase followed by a decrease). Initially (Day 2), the binding activity of the 36-kDa IGFBP increased as the degree of feed restriction increased. For instance, starvation resulted in a transitory increase in the 36-kDa IGFBP by Day 2 ($P < 0.05$). On Day 4, binding activity of the 36-kDa IGFBP appeared to be unaffected and this occurred in conjunction with an absence in effect on the plasma concentrations of IGF-I. This may suggest the formation of an IGF-I–36-kDa IGFBP complex resulting in a transient saturation/equilibrium of this complex or the complimentary influence of the 30-kDa IGFBP. On Day 16, binding activity of the 36-kDa IGFBP was decreased by feed restriction. Both these increases and these decreases appeared to be dependent on the degree of feed restriction. Although an increase in binding activity of the 36-kDa IGFBP with starvation in chickens has previously been reported (19), the decrease observed by Day 16 with mild feed restriction was new and unexpected. This observation may be due to the longer duration of this study (16 vs 10 days; c.f., Ref. 19), the varying degrees of feed restriction, and/or the age of the chickens at the start of the study (14 vs 40 days old).

The 40-kDa binding protein was present in all the plasma samples collected but was not influenced by feed restriction as the 30- or 36-kDa IGFBPs were (Table V). It is possible that a homology exists between this 40-kDa protein and mammalian IGFBP-3, since neither appear to be greatly affected by metabolic status (46). In addition, the mammalian IGFBP-3 has a molecular weight similar (approximately 40 kDa) to that of the 40-kDa IGFBP found in chickens (46).

In conclusion, we have shown that IGF-II plasma concentrations in chickens are clearly effected by nutritional regulation, but that a longer duration of feed restriction appears to be required to elicit a response. IGF-I binding activity is influenced by feed restriction and is accompanied by reductions in plasma concentrations of IGF-I and IGF-II and thereby decreases the availability of IGF-I and II. The level of IGFBP binding activity is regulated by varying the degree of feed restriction in a manner independent of IGF-I and II. The increased and decreased binding activities of the IGFBPs isolated in chickens, in conjunction with the IGF-I effects observed, provide greater insight into the mechanisms of IGF-I regulation. The bioavailability of IGF-I is obviously influenced by the binding of these IGFBPs, as evidenced by the reductions in plasma concentrations of IGF-I. We have also demonstrated that the plasma concentrations of IGF-I and IGF-II, and the binding activity of their related IGFBPs, can be influenced directly through feed restriction. This information could provide some insight into the management of malnutrition in both animals and humans. Of equal importance, the findings of this study suggest homologies between mammalian and chicken IGFBPs (i.e., mammalian IGFBP-1 and IGFBP-3 share similar characteristics in respect to dietary influence with the 30- and 40-kDa IGFBPs in chickens, respectively). The abundance of binding activity of the 40-kDa IGFBP suggests that it is one of the primary chicken IGFBPs, and that its role in IGF-I regulation warrants further study. The reductions in growth parameters (body weight and bone length) observed in this experiment, in conjunction with reductions in IGF-I concentrations, clearly demonstrate the importance of IGF-I in normal growth. The mechanisms by which IGFBPs regulate IGF-I intracellular and extracellular bioavailability are cause for further investigation.

1. Phillips LS, Vassilopoulou-Sellin R. Somatomedins. N Engl J Med **302**:371–380, 1980.
2. McCusker RH, Campion DR, Jones WK, Clemmons DR. The insulin-

like growth factor–binding proteins of porcine serum: Endocrine and nutritional regulation. Endocrinology **125:**501–509, 1989.
3. Hoshino S, Wakita M, Suzuki M, Yamamoto K. Effect of fasting on the levels of glucose, free fatty acids, growth hormone, and somatomedin in the serum and on pituitary function of the chicken. Jpn Poult Sci **17:**329–336, 1980.
4. Musey VC, Goldstein S, Farmer PK, Moore PB, Phillips LS. Differential regulation of IGF-1 and IGF-binding protein–1 by dietary composition in humans. Am J Med Sci **305:**131–138, 1993.
5. Yeoh SI, Baxter RC. Metabolic regulation of the growth hormone independent insulin-like growth factor binding protein in human plasma. Acta Endocrinol (Copenh) **119:**465–473, 1988.
6. Merimee TJ, Zapf J, Froesch ER. Insulin-like growth factors in the fed and fasted states. J Clin Endocrinol Metab **55:**999–1002, 1982.
7. Lauterio TJ, Scanes CG. Hormonal responses to protein restriction in two strains of chickens with different growth characteristics. J Nutr **117:**758–763, 1987.
8. Rosebrough RW, McMurtry JP, Mitchell AD, Steele NC. Chicken haptic metabolism *in vitro.* Protein and energy relations in the broiler chicken—VI. Effect of dietary protein and energy restriction on carbohydrate and lipid metabolism and metabolic hormone profiles. Comp Biochem Physiol **90B:**311–316, 1988.
9. Harvey S, Scanes CG, Chadwick A, Bolton NJ. Influence of fasting, glucose, and insulin on the levels in plasma of growth hormone and prolactin of domestic fowl. J Endocrinol **76:**501–506, 1978.
10. Maes M, Underwood LE, Ketelslegers JM. Plasma somatomedin-C in fasted and refed rats: Close relationship with changes in liver somatogenic but not lactogenic binding sites. J Endocrinol **97:**243–252, 1983.
11. Soliman AT, Hassan AE, Aref MK, Hintz RL, Rosenfeld RG, Rogol AD. Serum insulin-like growth factors I and II concentrations and growth hormone and insulin responses to arginine infusion in children with protein-energy malnutrition before and after nutritional rehabilitation. Pediatr Res **20:**1122–1130, 1986.
12. Vance ML, Hartman ML, Thorner MO. Growth hormone and nutrition. Horm Res **38:**85–88, 1992.
13. Eigenmann JE, De Bruijne JJ, Froesch ER. Insulin-like growth factor I and growth hormone in canine starvation. Acta Endocrinologica **108:**161–166, 1985.
14. Kita K, Tomas FM, Owens PC, Knowles SE, Forbes BE, Upton Z, Hughes R, Ballard FJ. Influence of nutrition on hepatic IGF-I mRNA levels and plasma concentrations of IGF-I and IGF-II in meat-type chickens. J Endocrinol **149:**181–190, 1996.
15. 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.
16. Francis GL, McMurtry JP, Johnson RJ, Ballard FJ. 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.
17. 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.
18. Lord APD, Bastian SEP, Read LC, Walton PE, Ballard FJ. 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.
19. 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.
20. McMurtry JP, Francis GL, 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.
21. 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.
22. Bolze MS, Osborne JM, Vecbastiks KA, White ME. Insulin-like growth factor binding proteins in rats respond to fasting and protein and energy content of the re-feeding diet. J Nutr Biochem **2:**623–628, 1991.
23. Clemmons DR, Seek MM, Underwood LE. Supplemental essential amino acids augment the somatomedin-C/insulin-like growth factor I response to refeeding after fasting. Metabolism **34:**391–395, 1985.
24. Takenaka A, Mori M, Yamada S, Ohgane J, Takahashi S-I, Noguchi T. Nutritional regulation of gene expression of insulin-like growth factor-binding proteins and the acid-labile subunit in various tissues in rats. J Endocrinol **150:**33–41, 1996.
25. Zapf J, Schmid C, Guler H-P, Binz K, Froesch ER. Effects of growth hormone, insulin, and IGF 1 treatment on IGF carrier proteins in hypophysectomized and diabetic rats. Comparison with fasting and refeeding. In: Drop SLS and Hintz RL. Eds. Insulin-Like Growth Factor Binding Proteins. Amsterdam: Elsevier Science Publisher BV, pp151–156, 1989.
26. Gallaher BW, Breier BH, Oliver MH, Harding JE, Gluckman PD. Ontogenic differences in the nutritional regulation of circulating IGF binding proteins in sheep plasma. Acta Endocrinologica **126:**49–54, 1992.
27. Gosiewska A, Wilson S, Kwon D, Peterkofsky B. Evidence for an in vivo role of insulin-like growth factor–binding protein–1 and –2 as inhibitors of collagen gene expression in vitamin C–deficient and fasted guinea pigs. Endocrinology **134:**1329–1339, 1994.
28. Peterkofsky B, Palka J, Wilson S, Takeda K, Shah V. Elevated activity of low molecular weight insulin-like growth factor–binding proteins in sera of vitamin C–deficient and fasted guinea pigs. Endocrinology **128:**1769–1779, 1991.
29. 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.
30. 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.
31. Buonomo FC, Grohs DL, Baile CA, Campion DR. Determination of circulating levels of insulin-like growth factor II (IGF-II) in swine. Domest Anim Endocrinol **5:**323–329, 1988.
32. 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.
33. Phillips LS, Goldstein S, Gavin JR III. Nutrition and somatomedin XVI: Somatomedin and somatomedin inhibitors in fasted and refed rats. Metabolism **37:**209–216, 1988.
34. Yang H, Cree TC, Schalch DS. Effect of a carbohydrate-restricted, calorie-reduced diet on the growth of young rats and on serum growth hormone, somatomedins, total thyroxine, and triiodothyronine, free T_4 index, and total corticosterone. Metabolism **36:**794–798, 1987.
35. Hoshino S, Wakita M, Kobayashi Y, Nishiguchi Y, Ozawa A, Hodate K, Kakegawa T, Suzuki M. Changes in serum concentrations of insulin-like growth factor–I, hormone and thyroid hormones in growing and fattening steers. J Anim Physiol Anim Nutr **65:**36–44, 1991.
36. Webster JR, Corson ID, Littlejohn RP, Stuart SK, Suttie JM. Effects of season and nutrition on growth hormone and insulin-like growth factor–I in male red deer. Endocrinology **13:**698–704, 1996.
37. Isley WL, Underwood LE, Clemmons DR. Dietary components that regulate serum somatomedin-C concentrations in humans. J Clin Invest **71:**175–182, 1983.
38. Thissen JP, Ketelslegers JM, Underwood LE. Nutritional regulation of the insulin-like growth factors. Endocrinol Rev **15:**80–101, 1994.
39. Hoshino S, Wakita M, Suzuki M, Yamamoto K. Changes in a somatomedin-like factor and immunoassayable growth hormone during

growth of normal and dwarf pullets and cockerels. Poult Sci **61:**777–784, 1982.
40. Straus DS, Takemoto CD. Specific decrease in liver insulin-like growth factor–I and brain insulin-like growth factor–II gene expression in energy-restricted rats. J Nutr **121:**1279–1286, 1991.
41. Dehoff MH, Stoner CS, Bazer FW, Collier RJ, Kraeling RR, Buonomo FC. Temporal changes in steroids, prolactin, and growth hormone in pregnant and pseudopregnant gilt during mammogenesis and lactogenesis. Domest Anim Endocrinol **3:**95–105, 1986.
42. Buonomo FC, Lauterio TJ, Baile CA, Campion DR. Determination of insulin-like growth factor I (IGF-I) and binding protein levels in swine. Domest Anim Endocrinol **4:**23–31, 1987.
43. Davenport ML, Svoboda ME, Koerbe KL, Van Wyk JJ, Clemmons DR, Underwood LE. Serum concentrations of insulin-like growth factor II are not changed by short-term fasting and refeeding. J Clin Endocrinol Metabol **67:**1231–1236, 1988.
44. Morishita D, Wakita M, Hoshino S. Effects of hypophysectomy, feeding of propylthiouracil and thyroxine supplement on IGF-I binding activity of cockerel serum proteins. Proc Jpn Soc Comp Endocrinol **6:**59, 1991.
45. Baxter RC, Martin JL. Binding proteins for the insulin-like growth factors: Structure, regulation, and function. Prog Growth Factor Res **1:**49–68, 1989.
46. Umezawa T, Ohsawa Y, Miura Y, Kato H, Noguchi T. Effect of protein deprivation on insulin-like growth factor-binding proteins in rats. Br J Nutr **66:**105–116, 1991.