



The Effect of Insulin-Like Growth Factor-I (IGF-I) on Protein Turnover in the Meat-Type Chicken (*Gallus domesticus*)

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ABSTRACT. Insulin-like growth factor-I (IGF-I) effects on chicken growth and development are poorly understood. This study examined the effect of IGF-I on protein synthesis rates in various tissues in the male broiler chicken. At three weeks of age, osmotic minipumps were subcutaneously implanted in the scapular area. Chickens were infused with either chicken IGF-I (450 $\mu\text{g}/\text{kg}$ BW/day) or saline. After treatment for 5 days, the chickens received a flooding dose of [^3H]-phenylalanine, and were sacrificed 20 min later. Wing vein blood samples were taken at 0, 5, 10 and 20 min post-injection. The following tissues were removed and frozen for analysis: pectoralis muscle, gastrocnemius muscle, heart, liver, and small intestine. *In vivo* total protein synthesis measurements were made using the double-label technique. Contractile protein degradation was evaluated using intracellular free 3-methylhistidine concentrations in skeletal muscle. There were no significant differences in absolute or relative body growth rates over the treatment period. Skeletal muscle (pectoralis and gastrocnemius) weights were significantly decreased with IGF-I treatment, while heart weight was significantly increased. Plasma insulin levels were significantly lower in IGF-treated chickens compared to that in control birds. There was no effect of IGF-I on protein synthesis rates in any of the tissues examined. Intracellular free 3-methylhistidine concentrations were higher in both the gastrocnemius (17%) and pectoralis muscles (25%) of chickens treated with IGF-I. This data demonstrates that IGF-I may have an indirect effect to regulate muscle protein turnover rates. COMP BIOCHEM PHYSIOL 119C;1:75–80, 1998. © 1998 Elsevier Science Inc.

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INTRODUCTION

In view of the anabolic effects on nitrogen economy obtained after the administration of growth hormone in swine and other domestic species, there is interest in determining whether insulin-like growth factor-I (IGF-I), a mediator of the effects of GH during longitudinal growth, would act by promoting protein synthesis in chickens. It is well established that intracellular proteins are subject to substantial synthesis and degradation processes. Growth of specific tissues or the whole body is simply the reflection of the small differences between synthesis and degradation rates. *In vitro* studies show that IGF-I enhances protein synthesis both in the intact chick embryo (22) and in tissues derived from chick embryos (13,23). Similar effects have been observed in tissues derived from growing birds (4). Uncertainty exists

as to the mechanisms of action of IGF-I on protein metabolism *in vivo*. When administered *in vivo*, some researchers have shown IGF-I to elicit a decrease in plasma amino acids and serum urea nitrogen levels (5), and enhance nitrogen balance (3). Following low-doses of IGF-I, decreases in protein breakdown and protein oxidation have not been uniformly observed in rats and humans (5,11,15,29).

The metabolic effect of IGF-I, when administered to chickens, is not well understood. There are three reports on the effect of exogenous human IGF-I administration on growth and carcass composition in meat-type chickens (9,16,28). All studies to date have failed to demonstrate any effect of IGF-I on growth in chickens. In the first study, a human analog (N-Met) of IGF-I was injected intramuscularly (i.m.) daily. Huybrechts *et al.* (9) infused human IGF-I into broiler chicks and observed a decrease in the size of the abdominal fat pad with no change in growth rate. However, no change in total carcass lipid was observed even though feed efficiency was slightly improved. The authors suggested that IGF-I may function as a repartitioning agent in birds. Due to the uncertain actions of IGF-I in birds, the

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objective of this study was to determine the effect of chronic chicken IGF-I administration on protein turnover in selected tissues of rapidly growing broiler chickens.

MATERIALS AND METHODS

Animals

Three-week-old Shaver male broiler chickens, weighing 505 g, and with equivalent daily growth rates during the preceding two week period, were randomly assigned to the two treatment groups. Growth rates were calculated by graphing daily body weight against days and finding the slopes. Osmotic minipumps (Model 2001, ALZA Corp., Palo Alto, CA) were implanted subcutaneously in the scapular area under local anesthesia. Chickens were infused with either chicken IGF-I (450 $\mu\text{g}/\text{kg}$ bwt/day, $n = 9$) or saline ($n = 10$). The dose of IGF-I was chosen based on a preliminary study (personal communication, F. Tomas). Recombinant chicken IGF-I was purchased from GROPEP Pty., Ltd., Adelaide, Australia. Birds were maintained in a climatically controlled environment (23–25°C temp, 50% humidity), with free access to water and feed (standard broiler chick starter diet). Body weights were measured every other day to determine growth rate.

In Vivo Total Protein Synthesis Measurements

After 5 days of IGF-I treatment, *in vivo* fractional protein synthesis rates were measured according to the flooding-dose technique of Garlick and coworkers (6). The birds received a single i.p. injection of unlabeled L-phenylalanine (150 $\mu\text{mol}/100$ g bwt) combined with 200 $\mu\text{Ci}/100$ g bwt of L-[4- ^3H] phenylalanine (Amersham Corp., Arlington Heights, IL) to flood the precursor pools. Wing vein blood samples (1 ml) were withdrawn at 0, 5, 10 and 20 minutes post-injection to determine the plasma specific radioactivity (S.A.) of [^3H]phenylalanine. Immediately after the final blood sampling, the animals were sacrificed with a sodium pentobarbital overdose. Pectoralis muscle, gastrocnemius muscle, heart, liver, and a segment of the small intestine were quickly removed, weighed, and frozen in liquid nitrogen within 10 minutes of sacrifice. The samples were stored at -80°C for later analysis.

To determine the specific activity of phenylalanine in the plasma, a sample was deproteinized with 95% ethanol and centrifuged (10,000 \times g, 10 min at 4°C) in an Eppendorf microfuge, and the supernatant evaporated to dryness. The residue was dissolved in 50 mM Na_2CO_3 buffer (pH 10.0) and a 10 μl sample (containing approximately 500 pmol of phenylalanine) was incubated with 10 μl of 2 mM [^{14}C] dansyl chloride (8 dpm/pmol) (Amersham Corp., Arlington Heights, IL) for 30 min at 37°C in preparation for chromatography. To determine the specific activity of phenylalanine in the free amino acid pool, approximately 50 mg of

powdered tissue was homogenized in a low salt buffer (40 mM NaCl, 5 mM NaPO_4 (pH 7.0), 1 mM Mg, 0.1 mM dithiothreitol, and 0.1 mM EDTA) and centrifuged. The supernatant was treated in the same manner as the plasma above.

To measure the specific radioactivity of phenylalanine incorporated into total protein, tissues were analyzed using a modified method of Gregory and coworkers (8). Approximately 50 mg of frozen, powdered tissue was homogenized with a Polytron homogenizer in extraction solution containing 40 mM $\text{Na}_4\text{P}_2\text{O}_7$, 20 mM Na_2PO_4 , pH 7.0, and 0.5 mM EDTA (25 mg tissue/ml of extraction solution). Ten percent SDS was then added to the extraction solution to produce a final concentration of 1%. The mixture was heated in a boiling water bath for 3 min and centrifuged at 10,000 \times g for 15 min at room temperature. A 100- μl aliquot of supernatant was transferred to a 1.5 ml Eppendorf tube to which 1 ml of ice-cold 10% trichloroacetic acid (TCA) was added to precipitate the protein. After standing overnight at 4°C, the mixture was centrifuged and the pellet was washed twice in ice-cold 5% TCA and once in a mixture (50/50, v/v) of 95% ethanol/ether. The pellet was finally washed in acetone and air dried. The dried pellets were then transferred to 5 ml ampoules (Wheaton Science Products, Millville, NJ) to which 0.85 ml of 6 N HCL was added. The sample was hydrolyzed overnight at 110°C (14). The hydrolyzate was evaporated to dryness, dissolved in 0.85 ml of water, and then passed over a 0.6-ml column of Dowex AG 50-X4 resin (BioRad Laboratories, Hercules, CA) for the removal of amino acids. The amino acids were eluted with 25% (w/v) NH_4OH . The eluate was evaporated to dryness and the amino acids were redissolved in 1.0 ml of distilled water. An 0.8 ml aliquot of this solution was taken for radioactivity determination, and the remainder used for the determination of phenylalanine concentration by isotope dilution (1), and subsequent chromatography.

Thin layer chromatography on polyamide layer sheets (Schleicher & Schuell, Keene, NH) was used to separate the individual dansylated amino acids. Chromatograms were developed in the first dimension in water/formic acid (100:3, v/v) and in the second dimension in benzene/acetic acid (90:10, v/v). Dansyl-phenylalanine was separated from the other dansylated amino acids by identification with standards visualized under UV light. The dansyl-phenylalanine spot was cut out from the polyamide sheet and placed into 10 ml of scintillation fluid [4.7% NCS-II (Amersham Corp., Arlington Heights, IL), 4% Insta-Fluor (Packard Instrument Co., Meriden, CT) in toluene] for radioactivity determination. From the known specific radioactivity of [^{14}C]dansyl chloride and the $^3\text{H}/^{14}\text{C}$ ratio of dansyl-phenylalanine, the specific radioactivity of [^3H]phenylalanine was determined.

Fractional synthesis rates (k_s) for total protein was calculated as follows:

$$k_s(\%/day) = \frac{\text{S.A. of phenylalanine in protein (d.p.m./pmol)} \times 100}{\text{S.A. of phenylalanine in intracellular free pool (d.p.m./pmol)} \times \text{time}}$$

where "time" refers to the duration of the measurement period (from time of injection to the tissue removal). Rates of protein synthesis were expressed as the percent of the protein pool synthesized per day (k_s , %/day).

Intracellular Free 3-Methylhistidine Concentrations

Determination of N-methylhistidine concentrations in skeletal muscles was performed on perchloric acid supernatants of lyophilized muscle using the methods previously described (30). 3-ethylhistidine was used as an internal standard.

Plasma Analysis

Plasma glucose, uric acid, insulin and IGF-I levels were measured in the time 0 blood sample. Plasma glucose levels were measured with a YSI glucose analyzer. Plasma uric acid levels were determined by kits purchased from Sigma Chemical Co., St. Louis, MO. Plasma insulin and IGF-I were determined using previously validated radioimmunoassays (17,18).

Statistical Analysis

The statistical significance of the difference between treatment means was determined using Student's *t* test (26).

RESULTS

The growth curves over the 5 day treatment period for both groups were similar. The birds gained approximately 50 g bwt/day. There were no significant differences in absolute body weight following 5 days of IGF-I infusion (Table 1).

Plasma glucose levels were similar in controls and IGF-I treated groups (245 vs 238 mg/dl, respectively; Table 1). However, plasma insulin concentrations were significantly ($P < 0.02$) lower in IGF-treated chicks compared to that in saline-infused birds (Table 1). Plasma uric acid concentrations were unaffected by treatment with IGF-I (Table 1). Plasma IGF-I concentrations were doubled in the IGF-I treated group compared to the saline infused birds (17 vs 40 ng/ml, Table 1).

Skeletal muscle (pectoralis and gastrocnemius) weights were significantly lower than controls ($P < 0.05$) following 5 days of IGF-I treatment, while heart muscle weight increased ($P < 0.05$) in response to IGF-I (Table 1). Liver

TABLE 1. Effects of IGF-I (450 μ g/kg bwt/day) or saline treatment on growth and metabolic parameters in chickens

Parameter	Treatment	
	IGF-I	Saline
Final body wt (g)	758 \pm 9	757 \pm 10
Gastrocnemius muscle (g)	4.82 \pm 0.20*	5.68 \pm 0.11
Pectoralis muscle (g)	28.91 \pm 1.01*	31.86 \pm 0.81
Ventricular wt (g)	3.15 \pm 0.07*	2.58 \pm 0.10
Liver wt (g)	18.51 \pm 0.54	19.86 \pm 0.64
Plasma IGF-I (ng/ml)	40.3 \pm 2.9*	17.2 \pm 0.9
Plasma insulin (ng/ml)	2.02 \pm 0.37**	3.60 \pm 0.31
Plasma glucose (mg/dl)	238 \pm 5	245 \pm 8
Plasma uric acid (mg/100 ml)	4.54 \pm 0.39	5.22 \pm 0.42

Values represent mean \pm sem for IGF-I ($n = 9$) and saline ($n = 10$) treatment groups.

*Denotes treatment differences ($P < 0.05$).

**Denotes treatment differences ($P < 0.02$).

weights were not significantly different between groups (Table 1).

The specific activity (S.A.) of [3 H]phenylalanine in plasma reached a plateau level by 10 min (Fig. 1). There were no significant differences between treatment groups. The S.A. of [3 H]phenylalanine in the ventricular intracellular free pool was found to be equal to that of the final plasma sample (results not shown). The "flooding-dose" technique is believed to flood all intracellular and extracellular compartments with radiolabelled amino acid of uniform S.A., including the immediate precursor [3 H]phenylalanyl-tRNA (7).

There were no significant differences in the fractional protein synthesis rates between groups any of the tissues examined (Table 2). Contractile protein degradation was evaluated using intracellular free 3-methylhistidine concentrations in skeletal muscles. Intracellular free 3-methylhistidine levels were higher in both the gastrocnemius (17%, $P < 0.10$) and pectoralis muscles (25%, $P < 0.05$) of chickens treated with IGF-I (Table 3). Providing other pathways for 3-methylhistidine flux are unaffected by treatment, these values indicate an increased rate of muscle protein breakdown with IGF-I treatment.

DISCUSSION

The objective of this study was to determine the effect of IGF-I on protein turnover in the meat-type chicken. There were no significant differences in absolute body weight or relative body growth rates over the treatment period. Significant reductions in the growth rates of skeletal muscles were noted, while ventricular weight increased when compared to the untreated controls. There were no significant differences in the fractional protein synthesis rates between treatments in any groups of the tissues examined. However, fractional protein synthesis rates for some of the tissues were

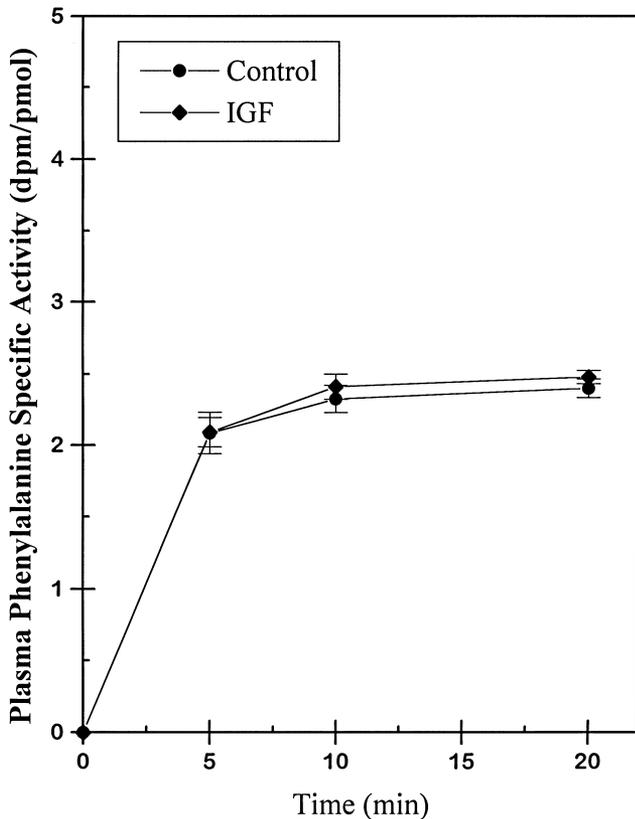


FIG. 1. S.A. of [^3H]phenylalanine in plasma. After injection of a "flooding" dose (150 $\mu\text{mol}/100\text{ g}$ body weight) of L-4- [^3H]phenylalanine, blood samples were taken at the beginning, middle, and end of the period of protein synthesis measurement, for the determination of the S.A. of the precursor [^3H]phenylalanine. (●) represents mean values at each time point for the control group and (◆) represents mean values at each time point for the IGF-I treated group. Error bars represent standard errors. Animals were injected at time 0.

similar to that previously reported (12,27). At 4 weeks of age, the body growth rates observed in this study and that previously reported (12,27) are similar (6–8%/day). Also, gastrocnemius muscle/leg protein synthesis rates at 4 weeks ranged from 17–24%/day, similar to that previously observed (12,27). However, our pectoralis muscle protein synthesis rates are much lower than those previously reported (6–8%/day vs 12–17%/day) (12,27). The intracellular free 3-methylhistidine values indicate an increase in skeletal muscle protein breakdown rates following IGF-I treatment. The mechanism for this is unknown. However, several studies have shown dramatic decreases in plasma amino acids and/or insulin levels with IGF-I treatment (2,10,11,15, 25,29). It has also been reported that in chickens, circulating insulin concentrations are depressed while uric acid levels are increased following a bolus i.v. injection of IGF-I (19). A similar effect was observed in this study in that plasma insulin concentrations were suppressed by IGF treatment. In humans, IGF-I suppresses the secretion of insulin

TABLE 2. Effect of IGF-I (450 $\mu\text{g}/\text{kg}$ bwt/day) or saline on tissue protein synthesis rates

Tissue	Fractional protein synthesis rate (K_s , %/Day) by treatment	
	IGF-I	Saline
Gastrocnemius muscle	23.33 \pm 2.84	17.00 \pm 2.12
Pectoralis muscle	5.98 \pm 0.67	8.43 \pm 1.03
Ventricular muscle	26.08 \pm 1.27	28.79 \pm 2.00
Liver	129.03 \pm 20.14	119.92 \pm 6.81
Small intestine	67.08 \pm 7.70	68.39 \pm 4.26

Values represent mean \pm sem for IGF-I ($n = 9$) and saline ($n = 10$) treatment groups.

by selective inhibition of detected secretory burst mass, whereas secretory burst frequency is not altered (24). This loss of substrate (*i.e.*, amino acid supply) could have caused an increase in skeletal muscle protein breakdown rates to replete plasma amino acid pools. In addition, the amounts of both insulin and free amino acid in muscle have been shown to affect the aggregation of subunits into active poly-ribosomes and the subsequent rate of protein synthesis (20). The same situation is seen when an animal is in a fasting or protein deficient state (21). Skeletal muscle may be the primary source of amino acids, while ventricular muscle is protected and can hypertrophy.

A lack of anabolic effect of IGF-I on muscle protein synthesis rates has also been reported by Jacob and coworkers (11). The authors concluded that the apparent lack of effect was caused by the concomitant suppression of both insulin and plasma amino acids that were produced by IGF-I treatment. However, when small doses of amino acids were administered with IGF-I, protein synthesis rates increased and insulin levels did not drop. Also, when insulin was administered in conjunction with IGF-I, protein synthesis rates increased despite the decrease in plasma amino acid levels. Sandstrom and coworkers (25) also concluded that their lack of effect of IGF-I in humans was due to a decrease in plasma amino acids with fasting and stress.

Insulin-like growth factor-I appears to have tissue specific effects on protein turnover in the chicken. White (pecto-

TABLE 3. Effect of IGF-I (450 $\mu\text{g}/\text{kg}$ bwt/day) or saline on concentrations of free 3-methylhistidine (nmol/g dry muscle) in skeletal muscle

Treatment	Treatment	
	IGF-I	Saline
Gastrocnemius muscle	173.87 \pm 9.02*	148.96 \pm 8.65
Pectoralis muscle	126.16 \pm 7.70**	101.02 \pm 6.69

Values represent mean \pm sem for IGF-I ($n = 9$) and saline ($n = 10$) treatment groups.

*Denotes treatment differences ($P < 0.10$).

**Denotes treatment differences ($P < 0.05$).

ralis) and red/mixed (gastrocnemius) muscle is affected differently as compared to cardiac muscle. The liver and small intestine appear to be resistant to the effects of chronic elevations in circulating IGF-I. The absence of an anabolic effect of IGF-I on protein synthesis may be due to the fact that since the meat-type chicken has been selected for high muscle growth rates, a "genetic ceiling" for muscle protein synthesis rates has been reached. For strains of chicken that have not been as intensively selected for muscle growth, there may be potential for enhancing muscle protein synthesis by exogenous IGF-I administration.

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