Response of Muscle Protein Synthesis to the Infusion of Insulin-like Growth Factor-I and Fasting in Young Chickens

K. Kita*, T. Shibata[2], M. Aman Yaman[1], K. Nagao and J. Okumura[2]

Laboratory of Grassland Science, University Farm, Graduate School of Bioagricultural Sciences, Nagoya University Togo, Aichi 470-0151, Japan

ABSTRACT : In order to elucidate the physiological function of circulating IGF-I on muscle protein synthesis in the chicken under malnutritional conditions, we administrated recombinant chicken IGF-I using a osmotic mini pump to fasted young chickens and measured the rate of muscle protein synthesis and plasma metabolite. The pumps delivered IGF-I at the rate of 22 μg/d {300 μg·(kg body weight·d)$^{-1}$}. Fractional rate of protein synthesis in the muscle was measured using a large dose injection of L-[2,6-^{3}H]phenylalanine. Constant infusion of chicken IGF-I did not affect plasma glucose level. Significant interaction between dietary treatment and IGF-I infusion was observed in plasma NEFA and total cholesterol concentrations. When chicks were fasted, IGF-I infusion decreased plasma NEFA and total cholesterol concentrations. On the other hand, IGF-I administration did not affect plasma levels of both metabolites. Fasting reduced plasma triglyceride concentration significantly. IGF-I infusion also decreased the level of plasma triglyceride. Plasma IGF-I concentration of young chickens was halved by fasting for 1 d. IGF-I infusion using an osmotic minipump for 1 d increased plasma IGF-I concentration in fasted chicks to the level of fed chicks. Fasting decreased body weight and the loss of body weight was significantly ameliorated by IGF-I infusion. There was a significant interaction between dietary treatment and IGF-I infusion in the fractional rate of breast muscle protein synthesis. There was no effect of IGF-I infusion on muscle protein synthesis in fed chicks. Muscle protein synthesis reduced by fasting was ameliorated by IGF-I infusion, but did not reach to the level of fed control. Muscle weight of fasted chicks infused with IGF-I was similar to fasted birds without IGF-I infusion, which suggests that muscle protein degradation would be increased by IGF-I infusion as well as protein synthesis in fasted chicks. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 12 : 1760-1764)***

Key Words : IGF-I infusion, Muscle, Protein Synthesis, Chickens

INTRODUCTION

Insulin-like growth factor-I (IGF-I) of chickens has been characterized and shown to consist of 70 amino acids (Ballard et al., 1990). As is found in mammals, the growth rate of chickens, which varies widely under various nutritional conditions, is highly related to the change in plasma IGF-I concentration (Rosebrough and McMurtry, 1993; Rosebrough et al., 1996; Kita et al., 1996b; 1997; Kita and Okumura, 1999). When chicks were fasted, plasma IGF-I concentration was decreased to the half of well-fed controls (M. Aman Yaman et al., 2000). As it is well established in many species that muscle protein synthesis is very sensitive to changes in nutritional conditions, an immediate fall in the rate of muscle protein synthesis was observed in fasted chickens (Kita et al., 1996a; M. Aman Yaman et al., 2000). Recently, it was found in mice that muscle protein synthesis reduced by fasting was increased by human IGF-I infusion (Bark et al., 1998). However, there has been little information as to the physiological function of IGF-I on muscle protein synthesis in the chicken under malnutritional conditions. In the present study, therefore, we administrated recombinant chicken IGF-I using a osmotic mini pump to fasted young chickens and measured the rate of muscle protein synthesis and plasma metabolite concentrations to elucidate the physiological function of circulating IGF-I in avian species.

* Corresponding Author: K. Kita. Tel: +81-5613-7-0203, Fax: +81-5613-7-0203, E-mail: kitak@agr.nagoya-u.ac.jp

[1] Laboratory of Animal Nutrition, Department of Animal Husbandry, Faculty of Agriculture, Syiah Kuala University, Darussalam, Banda Aceh 23111, Indonesia.

[2] Laboratory of Animal Nutrition, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan.

Received June 18, 2002; Accepted August 14, 2002

MATERIALS AND METHODS

Animals and experimental procedures

One hundred Single-Comb White Leghorn male chicks from a local hatchery (Hattori Yokei Ltd, Nagoya, Japan) were fed a commercial chick mash diet (CP 21.5%, ME 12.1 kJ/g) from hatching until 6 d of age in electrically heated brooders. At this age, 32 birds of uniform body weight (average initial body weight±SD was 77.3±2.1 g) were selected and divided evenly into 4 experimental groups of 8 birds each. The birds were placed in individual stainless steel metabolism cages in a temperature-controlled (29±1°C) room. Continuous illumination was provided. Sixteen chicks in 2 experimental groups were deprived of feeds. The 16 chicks in the remaining 2 groups were allowed free access to the commercial diet. After 1 d of

fasting, osmotic minipumps (Alzet model 2001D, Alza, Palo Alto, CA, USA.) were inserted s.c. in the back of 8 chicks in one of the fasted groups and 8 birds in one of fed groups. The pumps were filled with recombinant chicken IGF-I (GroPep Pty Ltd., Adelaide, SA, Australia) dissolved with acetic acid (0.1 mole/l). The pumps delivered IGF-I at the rate of 22 μg/d {300 μg·(kg body weight·d)$^{-1}$}. The remaining birds were used as intact controls. After 1 d of IGF-I infusion, the fractional rate of breast muscle protein synthesis was determined. Before birds were killed by cervical dislocation, blood was collected by heart puncture after light anesthesia with diethyl ether. Plasma was separated immediately and stored at -20℃ until analyses. Animal care was in compliance with the applicable guidelines of the Nagoya University Policy on Animal Care and Use.

Protein synthesis *in vivo*

Fractional rate of protein synthesis (Ks) in the muscle was measured using a large dose injection of L-[2,6-^{3}H]phenylalanine (12.3 MBq/mol, Amersham Japan; Tokyo, Japan) (Garlick et al., 1980). At 2 and 10 min after the injection, 4 birds each in each treatment were killed by cervical dislocation and breast muscle (pectoralis major) were removed, washed with physiological saline, blotted and weighed. Muscle samples were plunged into liquid nitrogen and then stored at -20°C until analyzed. Chemical analyses to measure the rate of protein synthesis of chickens was previously described by M. Aman Yaman et al. (2000).

Plasma IGF-I concentration

Plasma IGF-I concentration was determined by radioimmunoassay according to the method described by Ballard et al. (1990). Prior to the measurement of IGF-I concentration by radioimmunoassay, plasma was subjected to acid-ethanol extraction according to the method of Ballard et al. (1990) modified by Kita et al. (1996b). Recombinant chicken IGF-I and goat anti-rabbit immunoglobulin were purchased from GroPep Pty. Ltd. (Adelaide, SA, Australia). Chicken IGF-I was iodinated with carrier-free Na^{125}I (Amersham Japan, Tokyo, Japan) by a modification of the chloramine-T method as described by Read et al. (1986).

Plasma metabolite concentration

Plasma concentrations of glucose, NEFA, triglyceride and total cholesterol were measured by using commercial kits (glucose: Glucose C II-Test Wako; NEFA: NEFA C-Test Wako; triglyceride: Triglyceride G-Test Wako; total cholesterol: Cholesterol E-Test Wako; Wako Pure Chemical Co. Ltd., Osaka, Japan).

Statistics

Statistical analyses of data were performed by two-way ANOVA to test main and interactive effects of dietary treatment (fed vs. fasted) and IGF-infusion. All statistical analyses were performed using the General Linear Model Procedures (GLM; SAS/STAT Version 6, SAS Institute, Cary, NC, USA.). Differences between means were considered to be significant at $p<0.05$.

RESULTS

The influence of IGF-I infusion on plasma IGF-I concentration in fasted chicks is represented in Figure 1. Plasma IGF-I concentration was halved by fasting. When fed chicks were infused recombinant chicken IGF-I using an osmotic minipump, plasma IGF-I concentration was significantly higher than that of intact fed chicks. In fasted chicks, IGF-I infusion increased plasma IGF-I concentration to the level of fed chicks infused with IGF-I.

The influence of IGF-I infusion on body weight change, breast muscle weight and Ks of breast muscle of fasted chicks is shown in Table 1. Significant interaction between dietary treatment and IGF-I infusion was observed in body weight change. Fasting decreased body weight, and the loss of body weight was significantly ameliorated by IGF-I infusion. IGF-I infusion decreased body weight gain in fed chicks. Breast muscle weight in fasted chicks was almost half of that in fed chicks. There was a significant interaction between dietary treatment and IGF-I infusion in Ks. Fasting reduced Ks of breast muscle and the decrease in Ks was

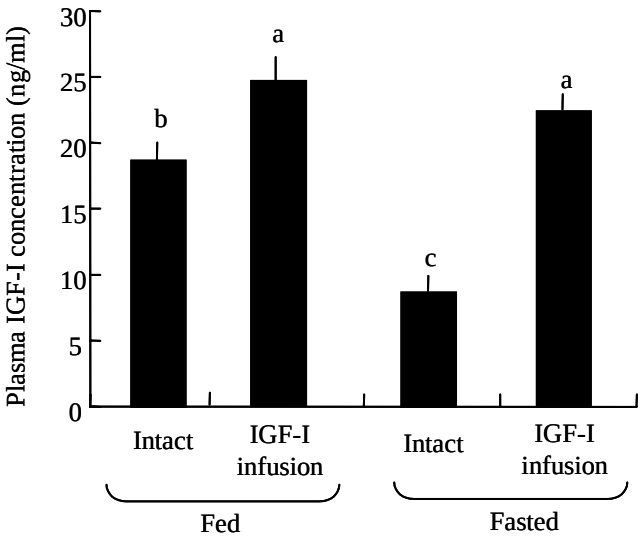

Figure 1. The influence of recombinant chicken IGF-I infusion on plasma IGF-I concentration in fasted chicks. Vertical bars represent means±SEM of 4 chicks. Means with different superscript letters were significantly different at $p<0.05$.

Table 1. Influence of insulin-like growth factor-I (IGF-I) infusion on body weight change, breast muscle weight and the fractional rate of breast muscle protein synthesis (Ks) of fasted chicks

		Body weight change (g)	Muscle weight (g)	Ks (%/d)
Fed	Intact	7.18±0.20[a]	2.30±0.23	15.4±0.78
	IGF-I infusion	5.25±0.80[b]	2.55±0.06	13.6±1.32
	Mean	6.21±0.53[a]	2.43±0.12[a]	14.5±0.79[a]
Fasted	Intact	-5.18±0.14[b]	1.40±0.06	4.9±0.61[b]
	IGF-I infusion	-3.50±0.36[a]	1.18±0.10	8.5±0.84[a]
	Mean	-4.34±0.36[b]	1.27±0.07[b]	7.0±0.90[b]
		Analysis of variance		
Diet		p<0.001	p<0.001	p<0.001
IGF-I infusion		p=0.789	p=0.933	p<0.368
Interaction		p=0.002	p=0.130	p=0.017

Chicks were fasted for 1 d and then an osmotic minipump was inserted s.c. in the back of chicks to deliver chicken IGF-I {300 μg·(kg body weight·d)$^{-1}$}. After 1 d of IGF-I infusion, Ks was determined by a large dose injection of L-[2,6-^{3}H]phenylalanine. Means±SEM. When the interaction was significant (p<0.05), the difference of means in the same dietary treatment was compared. Means with different superscript letters were significantly different at p<0.05.

ameliorated by IGF-I infusion. When fed chicks were infused with chicken IGF-I, muscle Ks was not changed.

Plasma concentrations of glucose, NEFA, triglyceride and total cholesterol are shown in Table 2. Constant infusion of chicken IGF-I did not affect plasma glucose level. Significant interaction between dietary treatment and IGF-I infusion was observed in plasma NEFA and total cholesterol concentrations. When chicks were fasted, IGF-I infusion decreased plasma NEFA and total cholesterol concentrations. On the other hand, IGF-I administration did not affect plasma levels of both metabolites. No interaction between dietary treatment and IGF-I infusion was observed in plasma triglyceride concentration. Fasting reduced plasma triglyceride concentration significantly. IGF-I infusion also decreased the level of plasma triglyceride.

DISCUSSION

As shown in Figure 1, plasma IGF-I concentration was significantly decreased by fasting, which is in good agreement with our previous results (Kita et al., 1997; M. Aman Yaman et al., 2000). In the present study, recombinant chicken IGF-I was infused by an osmotic minipump at the rate of 22 μg/d {300 μg·(kg body weight·d)$^{-1}$}, which successfully attained the normal level of plasma IGF-I concentration similar to intact controls.

In the present study, body weight was decreased by fasting and the loss of body weight was significantly ameliorated by IGF-I infusion (Table 1). Similar improvement of body weight was observed in energy-restricted rats which were infused human IGF-I (Schalch et al., 1989). However, IGF-I infusion was not effective to increase body weight gain in fed chicks. Several attempts have also been done to enhance the growth rate and carcass composition in well-fed chickens by the administration of exogenous IGF-I. However, the growth rate of young chickens was not improved by the administration of either recombinant human or chicken IGF-I (McGuinness and Cogburn, 1991; Huybrechts et al., 1992; Tixier-Boichard et al., 1992; Czerwinski et al., 1998). Similarly to avian

Table 2. Influence of insulin-like growth factor-I (IGF-I) infusion on plasma concentrations of glucose, non-esterified fatty acid (NEFA), triglyceride and total cholesterol in fasted chicks

		Glucose (mg/dL)	NEFA (mEq/L)	Triglyceride (mg/dL)	Total cholesterol (mg/dL)
Fed	Intact	265±8.4	0.86±0.03	101.1±14.1	169±6.4
	IGF-I infusion	244±4.9	0.73±0.05	70.3±9.0	131±4.5
	Mean	255±6.1[a]	0.80±0.04[a]	85.7±9.7[a]	150±8.1[a]
Fasted	Intact	229±15.6	0.86±0.11[a]	66.1±4.1	168±12.0[a]
	IGF-I infusion	214±4.3	0.43±0.04[b]	38.5±6.1	64±26.0[b]
	Mean	221±7.0[b]	0.62±0.10[b]	50.3±6.6[b]	108±24.4[b]
			Analysis of variance		
Diet		p=0.002	p=0.022	p=0.006	p=0.024
IGF-I infusion		p=0.055	p=0.003	p=0.013	p<0.001
Interaction		p=0.688	p=0.022	p=0.877	p=0.028

Chicks were fasted for 1 d and then an osmotic minipump was inserted s.c. in the back of chicks to deliver chicken IGF-I {300 μg·(kg body weight·d)$^{-1}$} for 1 d. Means±SEM. When the interaction was significant (p<0.05), the difference of means in the same dietary treatment was compared. Means with different superscript letters were significantly different at p<0.05.

species, IGF-I infusion could not attain the growth improvement in mammalian species under well-fed conditions (Schalch et al., 1989). Taken together, these results suggest that in both mammalian and avian species IGF-I infusion might be more effective in malnourished animals compared to well-fed animals.

Previously we reported that fasting reduced breast muscle Ks to about half of that in well-fed chicks (Kita et al., 1996a; M. Aman Yaman et al., 2000), and similar result was observed in the present study (Table 1). Although IGF-I infusion ameliorated the Ks decrease in fasted chickens, breast muscle weight of fasted chicks infused with IGF-I was similar to that without IGF-I infusion. As it has been well known that protein accumulation is resulted from the difference between protein synthesis and protein degradation (Kita et al., 1993), protein degradation in fasted chickens might also be stimulated by IGF-I infusion as well as protein synthesis. However, the mechanism of protein degradation stimulated by IGF-I infusion has not been studied in the present study and this issue should be investigated in the future.

As shown in Table 1, IGF-I infusion did not affect Ks in well-fed birds. The ineffectiveness of IGF-I administration for muscle protein synthesis of well-fed animals was also observed in rats (Jacob et al., 1996). These data suggest that IGF-I administration does not seem to be capable to stimulate muscle protein synthesis in well-fed animals including both mammalian and avian species. In contract to well-fed animals, there is inconsistency of the effect of IGF-I infusion on muscle protein synthesis in fasted animals. Bark et al. (1998) reported that IGF-I infusion was successfully able to increase muscle Ks reduced by fasting to the level of fed mice. As shown in Table 1, however, muscle Ks of fasted chickens was increased by IGF-I infusion but did not reach to the level of fed birds. In general, exogenous IGF-I administration to mammals brought about hypoglycemia because of insulin-like function of IGF-I (Jacob et al., 1989; Takano et al., 1991; Clemmons et al., 1992; Kerr et al., 1993; Kovacs et al., 1999). On the other hand, as it was recognized that in avian species the response of blood glucose to insulin is lower than that of mammalian species, blood glucose was not changed by IGF-I infusion in the present study (Table 2). As Waterlow et al. (1978) reviewed, protein synthesis is the energy-required process, and high-energy phosphate compounds like ATP and GTP should be supplied for stimulating muscle protein synthesis. Lower incorporation of blood glucose into muscular cells might not lead to produce high-energy phosphate compounds, resulting in failure to stimulate muscle protein synthesis.

We conclude that the reduction in muscle protein synthesis due to fasting is ameliorated by exogenous recombinant chicken IGF-I administration, but the increase in muscle protein synthesis does not contribute to the increment in muscle protein mass.

ACKNOWLEDGEMENT

This work was financially supported by Uehara Memorial Foundation, Tokyo, Japan. We were thankful to Dr. P. C. Owens (Cooperative Research Centre for Tissue Growth and Repair, Adelaide, SA, Australia) for the gift of rabbit polyclonal antisera against human IGF-I.

REFERENCES

Ballard, F. J., R. J. Johnson, P. C. Owens, G. L. Francis, F. Z. Upton, J. P. McMurtry and J. C. Wallace. 1990. Chicken insulin-like growth factor-I: Amino acid sequence, radioimmunoassay, and plasma levels between strains and during growth. Gen. Comp. Endocrinol. 79:459-468.

Bark, T. H., M. A. McNurlan, C. H. Lang and P. J. Garlick. 1998. Increased protein synthesis after acute IGF-I or insulin infusion is localized to muscle in mice. Am. J. Physiol. 275: E118-E123.

Clemmons, D. R., A. Smith-Banks and L. E. Underwood. 1992. Reversal of diet-induced catabolism by infusion of recombinant insulin-like growth factor-I in humans. J. Clin. Endocrinol. Metab. 75:234-238.

Czerwinski, S. M., J. M. Cate, G. Francis, F. Tomas, D. M. Brocht and J. P. McMurtry. 1998. The effect of insulin-like growth factor-I (IGF-I) on protein turnover in the meat-type chicken (*Gallus domesticus*). Comp. Biochem. Physiol. 119C:75-80.

Garlick, P. J., M. A. McNurlan and V. R. Preedy. 1980. A rapid and convenient technique for measuring the rate of protein synthesis in tissues by injection of [^{3}H]phenylalanine. Biochem. J. 192:719-723.

Huybrechts, L. M., E. Decuypere, J. Buyse, E. R. Kuhn and M. Tixier-Boichard. 1992. Effect of recombinant human insulin-like growth factor-I on weight gain, fat content, and hormonal parameters in broiler chickens. Poult. Sci. 71:181-187.

Jacob, R., E. Barrett, G. Plewe, K. D. Fagin and R. S. Sherwin. 1989. Acute effects of insulin-like growth factor I on glucose and amino acid metabolism in the awake fasted rat. Comparison with insulin. J. Clin. Invest. 83:1717-1723.

Jacob, B., X. Hu, D. Niederstock, S. Hasan, P. H. McNulty, R. S. Sherwin and L. H. Young. 1996. IGF-I stimulation of muscle protein synthesis in the awake rat: permissive role of insulin and amino acids. Am. J. Physiol. 270:E60-E66.

Kerr, D., W. V. Tamborlane, F. Rife and R. S. Sherwin. 1993. Effect of insulin-like growth factor-1 on the responses to and recognition of hypoglycemia in humans. A comparison with insulin. J. Clin. Invest. 91:141-147.

Kita, K., J. Iwata, T. Shibata, M. A. Conlon and J. Okumura. 1997. Influence of fasting on plasma insulin-like growth factor-I concentration in chickens at early stages of growth. Jpn. Poult. Sci. 34:410-413.

Kita, K., S. Matsunami and J. Okumura. 1996a. Relationship of protein synthesis to mRNA levels in the muscle of chicks under various nutritional conditions. J. Nutr. 126:1827-1832.

Kita, K., T. Muramatsu and J. Okumura. 1993. Effect of dietary protein and energy intakes on whole-body protein turnover and its contribution to heat production in chicks. Br. J. Nutr. 69:681-688.

Kita, K. and J. Okumura. 1999. Dietary protein levels alter plasma insulin-like growth factor-I concentration of chicks. Jpn. Poult. Sci. 36:25-30.

Kita, K., F. M. Tomas, P. C. Owens, S. E. Knowles, B. E. Forbes, Z. Upton, R. Hughes and F. J. Ballard. 1996b. 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.

Kovacs, G. T., S. Worgall, P. Schwalbach, T. Steichele, O. Mehls and L. Rosivall. 1999. Hypoglycemic effects of insulin-like growth factor-1 in experimental uremia: can concomitant growth hormone administration prevent this effect?. Horm. Res. 51:193-200.

Aman Yaman, M., K. Kita and J. Okumura. 2000. Various macronutrient intakes additively stimulate protein synthesis in liver and muscle of food-deprived chicks. J. Nutr. 130:70-76.

McGuinness, M. C. and L. A. Cogburn. 1991. Response of young broiler chickens to chronic injection of recombinant-derived human insulin-like growth factor-I. Domest. Anim. Endocrinol. 8:611-620.

Read, L. C., F. J. Ballard, G. L. Francis, R. C. Baxter, C. J. Bagley and J. C. Wallace. 1986. Comparative binding of bovine, human and rat insulin-like growth factors to membrane receptors and to antibodies against human insulin-like growth factor-I. Biochem. J. 233:215-221.

Rosebrough, R. W. and J. P. McMurtry. 1993. Protein and energy relationship in the broiler chicken. 11. Effects of protein quantity and quality on metabolism. Br. J. Nutr. 70:667-678.

Rosebrough, R. W., A. D. Mitchell and J. P. McMurtry. 1996. Dietary crude protein changes rapidly alter metabolism and plasma insulin-like growth factor I concentrations in broiler chickens. J. Nutr. 126:2888-2898.

Schalch, D. S., H. Yang, D. M. Ney and R. D. DiMarchi. 1989. Infusion of human insulin-like growth factor I (IGF-I) into malnourished rats reduces hepatic IGF-I mRNA abundance. Biochem. Biophys. Res. Comm.160:795-800.

Takano, K., N. Hizuka, K. Shizume, K. Asakawa, I. Fukuda and H. Demura. 1991. Repeated sc administration of recombinant human insulin-like growth factor I (IGF-I) to human subjects for 7 days. Growth Reg. 1:23-28.

Tixier-Boichard, M., L. M. Huybrechts, E. Decuypere, E. R. Kuhn, J. L. Monvoisin, G. Coquerelle, J. Charrier and J. Simon. 1992. Effects of insulin-like growth factor-I (IGF-I) infusion and dietary tri-iodothyronine (T_3) supplementation on growth, body composition and plasma hormone levels in sex-linked dwarf mutant and normal chickens. J. Endocrinol. 133:101-110.

Waterlow, J. C., P. J. Garlick and D. J. Millward. 1978. Protein turnover in the whole body and tissues. In: Protein turnover in mammalian tissues and in the whole body, North-Holland Amsterdam, pp. 626-695.

.

.