Effects of Refeeding with a Protein-Free Diets Supplemented with Various Essential Amino Acids on the Plasma Insulin-Like Growth Factor-I Concentration in Fasting Young Chickens

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ABSTRACT : The effect of refeeding with various single essential amino acids on the recovery of plasma insulin-like growth factor-I (IGF-I) concentration in fasted young chickens was examined. Young chickens (29 days of age) were divided into 15 experimental groups. Chickens in one group were fed on the commercial diet *ad libitum* for 4 days. The remaining 56 chickens in 14 experimental groups were fasted. After 2 days of fasting, 52 chicks in 13 fasted groups were refed with one of the following experimental diets for 2 days. Eleven experimental diets were protein-free diets supplemented with one of 11 essential amino acids (Arg, Gly, His, Ileu, Leu, Met, Phe, Lys, Thr, Trp, Val). The remaining 2 experimental diets were a protein-free diet containing 11 essential amino acids and a protein-free diet not supplemented with amino acids. Birds in the remaining fasted group continued to be fasted for 2 days. Fasting for 2 days markedly reduced plasma IGF-I concentration. When fasted chickens were refed the protein-free diet containing either Gly alone or all essential amino acids, plasma IGF-I concentration was recovered to the level similar to that of fed chickens. Protein-free diet alone, however, failed to restore the reduced IGF-I concentration in plasma. Body weight loss modulated by feeding with protein-free diets supplemented with various single essential amino acids was associated with changes in plasma IGF-I concentrations. We concluded that body weight loss by feeding with a protein-free diet was lower than that of fasted chickens and that body weight loss associated with the decrease in plasma IGF-I concentration was modulated by feeding with protein-free diets containing various single essential amino acids. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 3 : 406-409)***

Key Words : Essential Amino Acid, Fasting, Refeeding, Insulin-Like Growth Factor-I, 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 IGF-I. Its plasma concentrations vary with dietary protein and energy intakes in young chickens (Rosebrough et al., 1992a,b, 1996; Rosebrough and McMurtry, 1993). Fasting, for instance, reduced plasma IGF-I concentrations and it remained low until 24 h after refeeding and recovered to the level of fed control at 48 h after replenishment (Kita et al., 2002). In rats, Takenaka et al. (2000) reported that dietary restriction of single essential amino acids (Leu, Lys, Met, Thr) reduced plasma IGF-I concentration and that the effect of any single essential amino acid restriction was not significantly different from that of total amino acid deprivation. However, the effect of refeeding with single essential amino acids on the increase in plasma IGF-I concentration reduced by fasting has not been clarified.

In the present study, therefore, protein-free diets containing various single essential amino acids were given to fasting young chickens to elucidate the roles of dietary essential amino acids for regulating plasma IGF-I concentration.

MATERIALS AND METHODS

Two hundred day-old single-comb White Leghorn male chicks from a local hatchery (Hattori Yokei Ltd., Nagoya, Japan) were fed a commercial chick mash diet (crude protein 21.5%, metabolizable energy 12.1 kJ/g; Marubeni Shiryo Ltd., Tokyo, Japan) from hatching until 14 days of age in electrically heated brooders. A different type of commercial chick mash diet (crude protein 19.0%, metabolizable energy 11.8 kJ/g; Marubeni Shiryo Ltd., Tokyo, Japan) was offered from 15 to 29 days of age. At 29 days of age, 60 birds of uniform body weight (318.4±3.19 g) were selected and divided evenly into 15 experimental groups of 4 birds each. Chickens in one group were fed on the commercial diet *ad libitum* for 4 days. The remaining 56 chickens in 14 experimental groups were fasted for 2 days, then received 13 diet treatments, except for one group that

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remained fasting for an additional 2 days. Among treatments, eleven experimental diets were protein-free diets supplemented with one of 11 essential amino acids (Arg, Gly, His, Ileu, Leu, Met, Phe, Lys, Thr, Trp, Val). Remaining 2 experimental diets were a protein-free diet containing all 11 essential amino acids and a protein-free diet not supplemented with amino acids (PF). The level of amino acids supplemented into the protein-free diet satisfied the nutritional standard of the National Research Council (1984). The composition of experimental diets is shown in table 1. The birds were placed in individual stainless steel metabolism cages in a temperature-controlled (29±1°C) room. Continuous illumination was provided. Body weight and food intake were measured at the end of experimental period. Thereafter, blood was collected by heart puncture after light anesthesia with diethyl ether. Plasma was separated immediately and stored at -20°C until analyses. Then, birds were killed by cervical dislocation, and liver, breast muscle (*pectoralis major*) and leg muscle (*gastrocnemius*) were removed and weighed. Animal care was in compliance with applicable guidelines from the Nagoya University Policy on Animal Care and Use.

Plasma IGF-I concentration was measured by radioimmunoassay (RIA). Recombinant chicken IGF-I and goat anti-rabbit immunoglobulin were purchased from GroPep Pty. Ltd. (Adelaide, SA, Australia). Rabbit polyclonal antiserum against human IGF-I was a gift from Dr. P. Owens (Cooperative Research Centre for Tissue Growth and Repair, 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). Prior to the measurement of IGF-I concentration by RIA, plasma was subjected to acid-ethanol extraction according to the method described by Ballard et al. (1990) modified by Kita et al. (1996b).

Statistical analysis of data was performed by one-way ANOVA followed by Duncan's multiple range test (Duncan, 1955) using the General Linear Model Procedures (GLM; SAS/STAT Version 6, Statistical Analysis Systems Institute Inc., Cary, NC, USA.). Differences between means were considered to be significant at $p<0.05$. Regression equations were also calculated using GLM.

RESULTS

Body weight was markedly decreased by fasting, and body weight loss was ameliorated by refeeding with a protein-free diet compared to that of fasted chickens (table 2). The addition of various single essential amino acids into the protein-free diet did not significantly improve the amelioration. Body weight was increased to the level similar to the fed group by refeeding with the protein-free diet containing all essential amino acids.

Food intake of chickens given a commercial diet was the highest of all (table 2). When fasted chickens were refed with the protein-free diet containing all essential amino acids, food intake recovered to the same level as fed chickens. The influence of various single essential amino acids on food intake differed widely. Food intake of

Table 1. Composition of experimental diets (g/kg)

Amino acids	All	Arg	Gly	His	Ileu	Leu	Lys	Met	Phe	Thr	Try	Val	None
Ingredients													
L-arginine	10.0	10.0	-	-	-	-	-	-	-	-	-	-	-
Glycine	7.0	-	7.0	-	-	-	-	-	-	-	-	-	-
L-histidine	2.6	-	-	2.6	-	-	-	-	-	-	-	-	-
L-isoleucine	6.0	-	-	-	6.0	-	-	-	-	-	-	-	-
L-leucine	11.0	-	-	-	-	11.0	-	-	-	-	-	-	-
L-lysine·HCl	10.6	-	-	-	-	-	10.6	-	-	-	-	-	-
L-methionine	6.2	-	-	-	-	-	-	6.2	-	-	-	-	-
L-phenylalanine	10.0	-	-	-	-	-	-	-	10.0	-	-	-	-
L-threonine	6.8	-	-	-	-	-	-	-	-	6.8	-	-	-
L-tryptophan	1.7	-	-	-	-	-	-	-	-	-	1.7	-	-
L-valine	6.2	-	-	-	-	-	-	-	-	-	-	6.2	-
Cornstarch	674.6	742.7	745.7	750.1	746.7	741.7	742.1	746.5	742.7	745.9	751.0	746.5	752.7
Cellulose							154.3						
Corn oil							30.0						
Mineral mixture[1]							58.5						
Vitamin mixture[1]							2.0						
Choline chloride							1.5						
Inositol							1.0						

[1] Kita et al. (1996a).

Table 2. Influence of fasting followed by refeeding with single essential amino acids on body weight change, food intake, liver weight, breast muscle weight, leg muscle weight and plasma IGF-I concentration of young chickens[1]

	Body weight change (g) [2]	Food intake (g/2 days)	Liver weight (% of BW)	Breast muscle weight (% of BW)	Leg muscle weight (% of BW)	Plasma IGF-I (ng/mL)
Fed	16.6±5.3[a]	131.5±6.5*	2.73±0.18	3.45±0.13[a]	0.41±0.02	18.50±1.67[#]
EAA[3]	15.0±7.6[a]	128.6±10.8*	3.38±0.43	3.21±0.12[ab]	0.36±0.01	15.49±1.48[#]
Arg	-9.2±1.8[b]	68.7±16.0[†]	2.86±0.19	2.93±0.16[abc]	0.34±0.01	12.63±0.79[†]
Gly	-4.2±6.6[b]	80.0±22.4*[†]	2.86±0.37	2.82±0.16[bc]	0.33±0.04	15.53±0.47 [#]
His	-13.8±3.4[bc]	106.1±23.0*	2.69±0.10	2.64±0.26[bc]	0.34±0.02	10.58±0.76[†]
Ileu	-15.5±0.4[bc]	68.4±19.9[†]	2.48±0.16	2.97±0.12[abc]	0.34±0.01	13.87±0.64
Leu	-6.5±2.4[b]	97.3±8.3*	2.93±0.23	3.00±0.09[abc]	0.34±0.02	13.56±3.22
Lys	-17.9±5.0[bc]	41.0±22.0[†]	2.57±0.45	2.48±0.27[c]	0.31±0.01	11.62±0.82[†]
Met	-7.9±10.8[b]	107.6±14.0*	2.98±0.33	2.90±0.12[abc]	0.31±0.01	12.90±0.99[†]
Phe	-20.4±5.3[bc]	35.5±12.1[†]	2.35±0.18	3.14±0.07[ab]	0.30±0.02	12.25±1.21[†]
Thr	-19.2±3.2[bc]	94.8±18.2*[†]	2.29±0.04	3.02±0.07[abc]	0.34±0.02	9.35±0.33[†]
Trp	-5.9±9.3[b]	87.3±14.8*[†]	2.70±0.28	2.66±0.21[bc]	0.32±0.02	8.98±1.24[†]
Val	-13.0±3.4[bc]	65.6±14.6[†]	2.77±0.13	2.70±0.31[bc]	0.33±0.03	9.99±1.23[†]
PF [4]	-6.5±4.0[b]	89.2±24.3*[†]	2.99±0.37	3.04±0.15[abc]	0.31±0.02	10.88±1.13[†]
Fasted	-30.5±2.0[c]	-	2.19±0.05	2.63±0.21[bc]	0.33±0.02	9.59±1.57[†]

[1] The number of birds used was 4.
[2] Means±SEM.
[3] EAA, all essential amino acids.
[4] PF, protein-free.
Means within the same column with different superscript letters are significantly different at $p<0.05$. † Significantly different from fed chickens ($p<0.05$). * Significantly different from chickens fed the protein-free diet containing Phe ($p<0.05$). # Significantly different from fasted chickens ($p<0.05$).

chickens refed the protein-free diet containing methionine was three times higher than that in the phenylalanine-supplemented group.

When chickens were fasted, breast muscle weight was decreased (table 2). Refeeding with any of the experimental diets did not affect breast muscle weight compared to that of fasted chickens.

Liver and leg muscle, weights were not influenced by fasting and refeeding with any of the experimental diets (table 2).

Fasting markedly reduced plasma IGF-I concentration (table 2). When fasted chickens were refed the protein-free diet containing either all essential amino acids or Gly alone, plasma IGF-I concentration recovered to the level of fed chickens. Feeding with the protein-free diet, however, did not elevate the reduced plasma IGF-I concentration. The response of plasma IGF-I concentration to the addition of single essential amino acids into the protein-free diet differed widely.

DISCUSSION

As shown in table 2, fasting decreased body weight, and refeeding with a protein-free diet significantly ameliorated the loss of body weight. When chicks were fed a protein-free diet containing Met for 10 days, body weight loss was decreased compared to that of chicks given the protein-free diet alone (Muramatsu et al., 1986). In the present study, young chickens were fasted and then refed protein-free diets supplemented with various single essential amino acids for 2 days, and no significant improvement was observed in body weight loss. The discrepancy between these findings may be a result of the differences in age, feeding period or preliminary fasting before refeeding with experimental diets.

In the present study, food intake of chickens given the protein-free diet containing Phe alone was the lowest of all (table 2). When Phe was added into the low protein diet (10% of crude protein) from 1% to 5%, both food intake and body weight gain of chicks decreased with increasing dietary Phe levels (Okumura et al., 1980). These results suggest that imbalance of amino acids including Phe causes the inhibition of feeding behavior. We have previously reported that when chicks were fed the Phe excess diet, food intake was reduced by decreasing crop emptying rate (Furuse et al., 1991). In this report, it was also found that the elevation of plasma level of cholecystokinin, which has been suggested to be a satiety hormone, was elevated. Therefore, the reduction in food intake of chickens given other single essential amino acids may be a result of an increase in plasma cholecystokinin concentration accompanied by a decrease in crop emptying rate.

As shown in table 2, when fasted chickens were refed the protein-free diet containing either Gly alone or all essential amino acids, plasma IGF-I concentration recovered to the level of fed chickens. The influence of various single essential amino acids on plasma IGF-I concentration varied widely, though a significant difference was not observed. As we have previously reported, body weight gain is highly correlated to the change in plasma IGF-I concentrations in young chickens (Kita et al., 1996b; Kita and Okumura, 1999). Therefore, regression analysis was performed to examine the relationship between body weight change and plasma IGF-I concentration. The regression equation derived in the present study was: Body weight change (g/2 days)=-25.4+1.30 plasma IGF-I (ng/ml) ($r=0.29$, $p<0.05$). This relationship suggests that body weight loss modulated by feeding with protein-free diets supplemented with various single essential amino acids was associated with changes in plasma IGF-I concentrations.

We concluded that body weight loss by feeding with a protein-free diet was lower than that of fasted chickens and that body weight loss associated with the decrease in plasma IGF-I concentration was modulated by feeding with protein-free diets containing various single essential amino acids.

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