Influence of Refeeding of Protein, Carbohydrate and Fat on Hepatic Insulin-Like Growth Factor-I mRNA Level in Fasted Chicks

K. Kita[1], K. Hangsanet and J. Okumura
School of Agricultural Sciences, Nagoya University, Chikusa-ku, Nagoya 464-01, Japan

ABSTRACT : The influence of refeeding either protein, carbohydrate or fat on hepatic insulin-like growth factor-I (IGF-I) mRNA level in chicks which had been fasted for 2 days was examined. The hepatic IGF-I mRNA was measured by ribonuclease protection assay. Fasting reduced hepatic IGF-I mRNA levels to less than half of those in the fed control.

When chicks were refed either a control, protein or carbohydrate diet, IGF-I mRNA levels significantly increased to those in the fed control until 2 hours of refeeding. Refeeding of fat did not alter hepatic IGF-I mRNA levels. The significant correlation between liver weight and hepatic IGF-I gene expression suggests that when chicks are refed after 2-d fasting, the acute increase in hepatic IGF-I gene expression brought about after refeeding may be partly regulated by the increase in liver protein metabolism.

(Key Words: Chicken, IGF-I, Liver, Refeeding, Nutrient)

INTRODUCTION

The insulin-like growth factor-I (IGF-I) has been purified from chicken serum and characterized. It consists of 70 amino acids (Ballard et al., 1990). Several studies provide evidence for ontogenetic changes in plasma IGF-I concentration and hepatic IGF-I mRNA level in chickens.

After hatching, plasma concentration of IGF-I increases rapidly with advancing age, reaches a peak around 4 to 8 weeks of age, and then declines gradually (Johnson et al., 1990; Kikuchi et al., 1991; McGuinness and Cogburn, 1990). We have examined the alteration in hepatic IGF-I mRNA levels after hatching and showed that IGF-I mRNA levels in the liver significantly increased from 0 to 1 week of age, and thereafter the level tended to increase toward 8 weeks of age (Kita et al., 1997).

It has been well known that hepatic IGF-I mRNA level is very sensitive to change in nutritional status. Our previous findings have revealed that hepatic IGF-I gene expression in the chicken is responsive to alterations in nutritional conditions (Kita et al., 1996b). In this report, hepatic IGF-I mRNA levels are reduced by restriction of food intake and are reversed by refeeding. These results suggest that nutrition is one of important factors to regulate hepatic IGF-I gene expression in chickens during growing stages. However, the influence of each nutrient, protein, carbohydrate and fat on hepatic IGF-I gene expression has not been examined so far. Therefore, in the present study, we have examined the influence of refeeding of each nutrient on hepatic IGF-I mRNA levels in fasted chickens.

MATERIALS AND METHODS

Two hundreds single-comb White Leghorn male chicks from a local hatchery (Hattori Yokei Ltd, Nagoya, Japan) were fed on a commercial chick mash diet (Pre-Chick®, crude protein 210 g/kg, metabolizable energy 11.8 kJ/g; Marubeni Siryou Ltd, Tokyo, Japan) from hatching until 2 weeks of age in stainless steel cages in a temperature-controlled (29±1℃) room. Continuous illumination was provided. From 2 to 6 weeks of age, chicks were given different type of commercial chick mash diet (Chick-15®, crude protein 190 g/kg, metabolizable energy 11.8 kJ/g; Marubeni Siryou Ltd, Tokyo, Japan). At 6 weeks of age, 30 birds of uniform body weight were selected and divided evenly into 6 experimental groups of 5 birds each.

Twenty-five chicks in 5 experimental groups were fasted. The remaining group had free access. After 2 days of fasting, fasted chicks were divided into 4 experimental groups and refed the experimental diets. The composition of experimental diet was shown in table 1. Five chicks in one of the fasted groups were force-fed 23.5 g of the control diet mixed with water. The amount of the diet,

[1] Address reprint requests to K. Kita.
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Table 1. Composition of experimental diets

Ingredients	Complete	Carbohydrate	Protein	Fat
	g/kg diet			
Isolated soybean protein[1]	239.0	–	966.4	–
L-Methionine	2.9	–	11.7	–
L-Threonine	1.2	–	4.9	–
Glycine	4.2	–	17.0	–
Corn starch	302.0	457.8	–	–
Sucrose	200.0	303.2	–	–
Cellulose	157.7	239.0	–	–
Corn oil	30.0	–	–	1,000.0
Mineral mixture[2]	58.5	–	–	–
Vitamin mixture[2]	2.0	–	–	–
Choline chloride	1.5	–	–	–
Inositol	1.0	–	–	–
Crude protein (g/kg)	200.0	0	811.8	0
Metabolizable energy (MJ/kg)	12.6	15.3	14.8	37.3
Feeding level (g/bird)	23.5	15.5	5.8	0.7

[1] Crude protein content was 840 g/kg.
[2] Kita et al., (1996a).

23.5 g, was the maximum amount of the control diet which could be force-fed into the crop of chicks at once. Fifteen unfed birds in the 3 other treatment groups were force-fed one of the experimental diets containing only protein, carbohydrate or fat. The amounts of each experimental diet force-fed with water were 5.8, 15.5 and 0.7 g of the protein, carbohydrate and fat diets, respectively. These amounts corresponded to the equivalent level of each nutrient in 23.5 g of the control diet. The remaining 5 fasted chicks were used as non-fed controls. At 2 hours after refeeding, chicks were killed by cervical dislocation and livers were removed, washed with physiological saline, blotted and weighed. Liver samples were immediately frozen into liquid nitrogen and then stored at −80℃ until analyzed. Animal care was in compliance with the applicable guidelines of the Nagoya University Policy on Animal Care and Use.

Total RNA was extracted from liver samples according to the acid guanidine thiocyanate-phenol-chloroform method (Chomczynski & Sacchi, 1987). To detect hepatic IGF-I and β-actin mRNA, a ribonuclease protection assay was performed by using an RNA II Ribonuclease Protection Assay Kit (Ambion Inc., Austin, TX, U.S.A.), according to the procedure described previously (Kita et al., 1996b). In brief, Chicken IGF-I cDNA was generously donated by Dr. P. Rotwein (Washington University School of Medicine, St. Louis, MO, U.S.A.). Chicken β-actin cDNA was generously gifted by Dr. S. Dogra (University of Adelaide, Adelaide, SA, Australia). Both cDNAs were subcloned into plasmid pSP73 (Bresatec, Adelaide, SA, Australia) for generating labelled antisense riboprobe using SP6 and T7 polymerases according to the protocol in an *in vitro* transcription kit (Bresatec, Adelaide, SA, Australia). In this assay, 10 μg of RNA samples was used. The intensity of protected chicken IGF-I mRNA (329 bp) and β-actin mRNA (251 bp) bands were measured using a bio-imaging analysis system (BAS 2000, Fuji Photo Film, Co. Ltd., Tokyo, Japan).

Data was analysed by one-way analysis of variance to assess the significance of the effects of nutritional treatment. The Duncan's multiple range test was performed to compare between all pairs of means using the General Linear Model procedure (GLM; SAS/STAT Version 6) of a commercial statistical package, SAS (SAS Institute, Cary, NC, U.S.A.).

RESULTS

Fasting for 2 days significantly decreased body and liver weights, and refeeding of experimental diets tested in the present study did not alter body weight within 2 hours (table 2). Although refeeding with lipid diet did not change liver weight, liver weights of chicks refed with either complete, protein or carbohydrate diet were heavier than those in the fasted group.

Tabel 2. Body and liver weights of chicks refed either protein, carbohydrate or fat after 2 days of fasting

Nutritional conditions	Body weight (g)	Liver weight (g)
Fed	533[a]	13.8[a]
Fasted	444[b]	9.2[d]
Refed		
Complete	449[b]	11.7[b]
Protein	429[b]	10.2[cd]
Carbohydrate	447[b]	11.1[bc]
Fat	448[b]	9.3[d]
Pooled SEM	8.7	0.39

The number of chicks used was five per treatment.
Means in a column not sharing a superscript letter are significantly different, $p < 0.05$.

The IGF-I mRNA level in the liver decreased significantly after 2 days of fasting and was returned to the level of fed chicks by refeeding either complete, protein or carbohydrate diet (figure 1). The hepatic IGF-I mRNA level reduced by fasting did not increase following refeeding with fat.

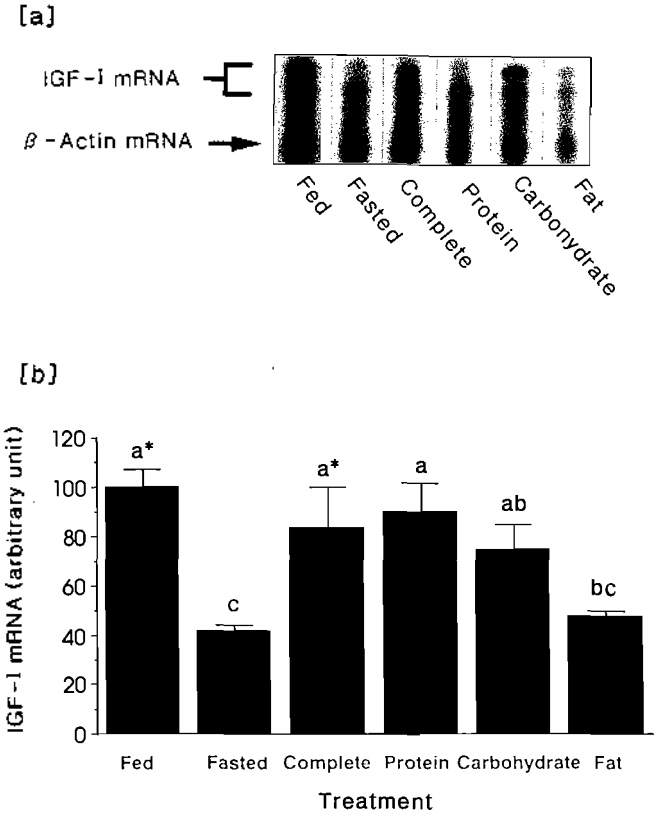

Figure 1. [a] Hepatic IGF-I and β-actin mRNA detected by ribonuclease protection assay. [b] IGF-I mRNA levels in the liver of chicks refed either protein, carbohydrate or fat after 2 days of fasting. Means ± SEM (n = 5). Means not sharing the same letter are significantly different at $p<0.05$. *, One missing value.

DISCUSSION

As shown in figure 1, IGF-I mRNA level was halved by 2-d fasting, and this reduction is consistent with other studies investigating the influence of malnutrition on IGF-I gene expression in chickens (Kita et al., 1996b), rats (Hayden et al., 1994; Kanamoto et al., 1994; Lemozy et al., 1994) and heifers (Vandehaar et al., 1995). These studies suggest that hepatic IGF-I gene expression is reduced by malnutrition in mammalian and avian species. However, the acute influences of refeeding with individual nutrient on hepatic IGF-I mRNA gene expression in fasted animals had not been examined to date. In the present study, we have shown that hepatic IGF-I gene expression was fully recovered within 2 hours of refeeding in chickens, and that refeeding protein only stimulated to increase IGF-I mRNA to the level of fed control.

As shown in table 1, liver weights of chicks refed with either complete, protein or carbohydrate diet were heavier than those in the fasted group, though refeeding with lipid diet did not change liver weight, which was in good agreement with our previous report (Kita et al., 1996a). As represented in figure 2, the regression analysis indicates the significant correlation between liver weight and the intensity of hepatic IGF-I mRNA levels. The regression equation which we derived was as follows:

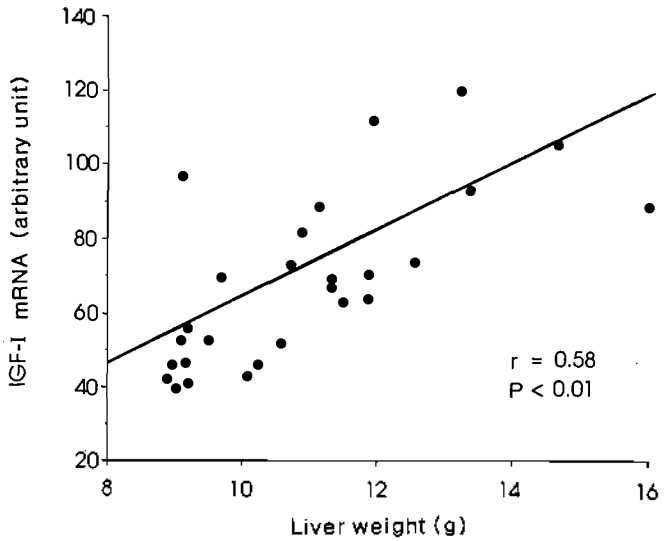

Figure 2. Relationship between liver weight and hepatic IGF-I mRNA levels in chicks refed either protein, carbohydrate or fat after 2 days of fasting.

Hepatic IGF-I mRNA (arbitrary unit)
= $-24.8 + 8.9$ Liver weight (g) ($r = 0.58$, $p < 0.01$)

We have previously reported that the acute recovery of liver weight in chicks refed with experimental diets was associated with the increase in the rate of liver protein synthesis. Therefore, this equation suggests that when chicks were refed after 2-d fasting, acute increase in hepatic IGF-I mRNA level brought about after refeeding may be partly regulated by the alteration in liver protein synthesis.

These results suggest that hepatic IGF-I gene expression in young chickens was very sensitive to fasting and refeeding with individual nutrients, and changes in hepatic IGF-I gene expression may associate with changes in liver protein metabolism.

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