Protein nutrition and insulin-like growth factor system

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Protein metabolism of growing animals is greatly affected by quantity and nutritional quality of dietary proteins. When animals are fed diets that contain enough proteins of good nutritional quality, they grow well. However, if they are fed diets deficient in protein or in some essential amino acids, their growth rate is markedly depressed. In this paper, we review the response of plasma insulin-like growth factor-I (IGF-I) to quantity and nutritional quality of dietary proteins. The sound correlation between plasma IGF-I concentration and the gain or loss of body proteins under various nutritional conditions suggests that the plasma IGF-I most possibly regulates the growth rate of animals or the rate of whole body protein synthesis. The quantity and nutritional quality of dietary proteins also regulates plasma concentration of IGF-binding proteins (IGFBPs). The changes in plasma concentration of IGFBPs presumably modifies the activity of IGF-I to regulate whole body protein synthesis. Molecular mechanisms of the changes in plasma concentrations of IGF-I and IGFBPs as affected by dietary proteins are also reviewed.

Protein nutrition: Essential amino acid deficiency: Insulin-like growth factor: Insulin-like growth factor binding protein: Insulin

Whole body protein has been shown to turn over at about 3 % per day in adults (for review, see Crim & Munro, 1994). This means that approximately 300 g of body protein is synthesized and, at the same time, degraded everyday. Synthesis and degradation of body proteins have been shown to be regulated by many factors including nutritional conditions, hormonal states and others.

Nutritional conditions affect the body protein metabolism extensively. Quantity and nutritional quality of dietary proteins regulate many metabolic activities in animals. If we are fed on diets deficient in protein or some essential amino acids, we lose body proteins and suffer from severe malnutrition. In those cases, decreases in some plasma proteins, e.g. serum albumin, pre-albumin and retinol-binding protein, and loss of many enzymes in liver and other tissues have been shown (for review, see Crim & Munro, 1994). Then, what kinds of factors regulate these changes in protein metabolism?

Effect of protein nutrition on plasma insulin concentration

Insulin has been known to be an anabolic hormone and has the activity to stimulate the storage of body proteins (for review, see Welle, 1999). Therefore, we investigated the relationship between the plasma insulin concentration and the body weight changes under various nutritional conditions. We used several kinds of diets. One was a casein diet (C diet with 120 g casein/kg diet). With this diet, young rats grew very well. On the other hand, when rats were fed on a wheat gluten diet (G diet, isonitrogenous with C diet) or a corn gluten meal diet supplemented with arginine (CGMA diet, isonitrogenous with C diet), they did not grow well. If the deficient amino acids, i.e. lysine and threonine to G diet, and lysine and tryptophan to CGMA diet, are supplemented to these diets, the rats grew as those fed on C diet. When rats were given a protein-free diet (PF diet), they lost body weight. These diets were given from 10:00 to 18:00 h everyday for 1 week. After 1 week, the rats were killed at 11:30 h. This means that the rats were fed on the diet for 1·5 h on the last day. Plasma immunoreactive insulin of these rats was determined. The results showed that plasma insulin concentration did not explain the difference in growth rate among the rats fed on the three diets of various nutritional value (data not shown). We also fed the rats diets of various casein content. The difference in the growth rate among the rats fed on those diets could not be explained by the difference in plasma

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Table 1. Effect of dietary proteins on plasma insulin-like growth factor-I (IGF-I) concentration in rats (Takahashi *et al.* 1990)

Diet	Final body weight (g)		IR-IGF-I (U/ml) (before acid–ethanol extraction)		Total IGF-I (U/ml) (after acid–ethanol extraction)	
	Mean	SE	Mean	SE	Mean	SE
PF	123·0[a]	1·2	1·61[a]	0·31	17·4[a]	1·4
G	133·6[b]	5·0	3·76[abc]	0·61	23·1[ab]	1·8
GLT	144·4[c]	3·4	4·53[bc]	0·56	19·3[a]	2·5
CGMA	135·8[b]	1·7	3·40[ab]	0·63	23·8[ab]	3·5
CGMALT	137·8[b]	2·5	5·83[cd]	1·15	25·8[ab]	2·6
C	153·0[d]	1·9	7·37[d]	0·98	30·7[b]	5·9

The initial body weight of the rats was 136·9 (SE 1·3) g.
Values are means with their standard errors for five rats per group.
[a,b,c,d] Values in columns with unlike superscript letters were significantly different ($P < 0.05$ or less) by Duncan's multiple range test.
Rats were fed on the diets containing casein (C diet with 120 g casein/kg diet), wheat gluten (G diet, isonitrogenous with C diet), G supplemented with lysine and threonine (GLT), corn gluten meal (CGMA, isonitrogenous with C diet), CGMA supplemented with lysine and tryptophan (CGMALT), and no protein (PF). After 1 week on these diets, blood samples were obtained from the carotid artery. IGF-I concentration in plasma was determined by radioimmunoassay.

insulin concentration. Then we tried to find another hormone which correlates with the difference in the growth rate of the rats fed on the diets of various nutritional value.

Effect of protein nutrition on plasma concentration of IGF-I

When we started the studies of the role of IGF-I in protein nutrition, IGF-I had been suggested to work as a protein anabolic hormone. The observations suggested that IGF-I is possibly a hormone that principally regulates body protein metabolism in many nutritional conditions. Then, we tried to elucidate whether the response of IGF-I under various nutritional conditions can explain the classical observations in protein nutrition.

In the series of experiments planned to elucidate the effect of dietary proteins on the plasma hormone concentrations, we have found that the plasma concentration of insulin-like growth factor (IGF)-I correlates well with the growth rate of young animals given the diets with the proteins of various nutritional values, see Table 1 (Takahashi *et al.* 1990). Feeding schedule was the same as that described earlier. The rats fed on the C diet grew very well and showed the high plasma IGF-I concentration. On the other hand, the rats fed on the PF diet, had very low plasma IGF-I concentration. The rats fed on the G diet showed the intermediate value in growth rate and plasma IGF-I concentration. The effect of casein content of diets also regulates the plasma concentration of IGF-I (Takahashi *et al*. 1990; Clemmons & Underwood, 1991). Then, we tried to elucidate the relationship between plasma IGF-I concentration and the whole body protein synthesis. The rate of whole body protein synthesis was determined by the 'massive dose method' proposed by Garlick *et al.* (1980). Plasma IGF-I concentration correlated with the rate of whole body protein synthesis (Nam *et al.* 1990). Later it was elucidated that if IGF-I is given to men with growth hormone resistance, it improves the growth rate (for review see Reiter & Rosenfeld, 1998). Thus, IGF-I is now widely believed to be one of the most important hormones which regulates growth rate or body protein metabolism.

IGF-I

IGF-I is a hormone the structure and function of which are similar to those of insulin (for review, see Noguchi *et al.* 1994). Many observations suggest that the physiological role of IGF-I is different from that of insulin. First, IGF-I is produced mainly in the liver. It is also produced locally in peripheral tissues. Second, in contrast to insulin, IGF-I is found in plasma bound to at least six kinds of specific binding proteins called IGF-binding proteins (IGFBPs). Third, the concentration of IGF-I is from 10 to 100 times higher than that of insulin. The plasma half-life of IGF-I is several hours whereas that of insulin is several minutes or a little longer. Fourth, plasma insulin concentration rises after a meal but that of IGF-I does not respond to a meal. On the contrary, plasma IGF-I concentration changes correlating to quantity and nutritional quality of dietary proteins, but insulin does not. IGF-I has its specific receptor, the IGF-I receptor, and insulin, the insulin receptor. They cross-react to each other. However, the binding constant is approximately 100 times lower than that of their own receptors. These observations suggest strongly the difference in the physiological role of insulin and IGF-I.

Changes in IGF-I mRNA in liver in response to dietary proteins

From the observations described, we tried to elucidate the difference in IGF-I mRNA content in liver (Miura *et al.* 1992). Liver IGF-I mRNA changed correlating to the plasma IGF-I concentration. We then studied the activity of the transcriptional rate of IGF-I gene by nuclear run-on assay. However, there was only about 30 % difference in the transcriptional rate between the rats fed on C and PF diet (data not shown). We concluded that this difference is not the principal reason of the difference in the IGF-I mRNA content in liver. Probably, the stability of IGF-I mRNA regulates the mRNA content in liver under various nutritional conditions.
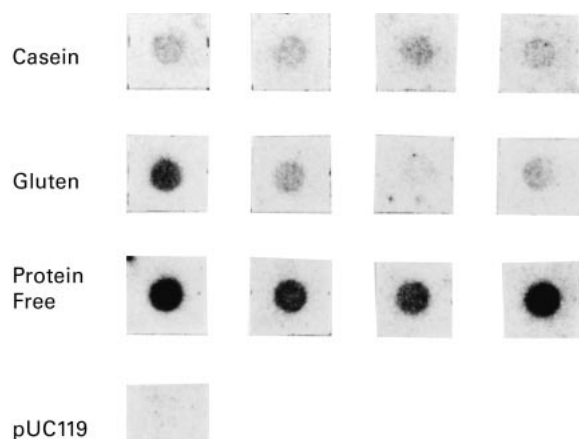
Fig. 1. Effect of C, G and PF diets on transcription rate of IGFBP-1 gene in rat liver (Miura *et al.* 1993). Rats were fed on C, G and PF diets. For diets, see Table 1. After 1 week on these diets, transcriptional rate of IGFBP-1 gene was determined by nuclear run-on assay. Each spot shows the result of nuclear run-on assay for one rat on the dies shown on the left. pUC119 is the vector.

Effect of protein nutrition on plasma concentration of IGFBPs

Next, we investigated the effect of protein nutrition on plasma IGFBP concentrations.

The results showed that plasma concentration of IGFBP-3 changed in parallel to that of IGF-I (for review, see Noguchi *et al.* 1994). On the other hand, plasma IGFBP-1 and IGFBP-2 concentrations increased extensively when the rats were given a protein-free diet or diets deficient in some essential amino acids.

IGFBPs

Six IGFBPs and several IGFBP-related proteins have been characterized (for review, see Noguchi *et al.* 1994 and Ooi & Boisclair, 1999). These IGFBPs have homology of amino acid sequence in N-terminal and C-terminal regions of their structure as reviewed by Ooi & Boisclair (1999). However, these IGFBPs have a different amino acid sequence in the central domain. Physiological roles of these IGFBPs have not been well elucidated. However,

studies on the effect of these IGFBPs on the action of IGF-I to cells suggest that IGFBPs modulate the activity of IGF-I action. Other possibilities that IGFBPs work as storages of IGF-I or that they elongate the life span of IGF-I in plasma are also proposed. Studies using transgenic mice suggest that some IGFBPs inhibit the activity of IGF-I. Some IGFBPs are also known to have their own biological activity independent of IGF-I.

Effect of protein nutrition on the mRNA content of IGFBPs in liver

In order to elucidate the mechanism of the effect of protein nutrition on plasma IGFBPs, we studied the mRNA content of IGFBPs in liver. Correlating to the increase in plasma IGFBP-1 concentration under protein malnutrition, a significant increase in IGFBP-1 mRNA was observed in liver. Plasma IGFBP-2 concentration also increased in protein deficiency and IGFBP-2 mRNA increased in liver. Plasma IGFBP-3 concentration decreased greatly in protein deficiency. However, this decrease did not correlate with the mRNA content of IGFBP-3 in liver.

Among these IGFBPs, the increase in IGFBP-1 mRNA was very marked (Takenaka *et al.* 1993). The content of IGFBP-1 mRNA in liver has been known to be affected also by insulin and glucocorticoids. Insulin decreases the content, but glucocorticoids increase it. We studied whether the increase in IGFBP-1 mRNA in protein deficiency is mediated by insulin or glucocorticoid. The results suggested that it is not mediated by these hormones because we could identify an amino acid responsive element in the promoter region of IGFBP-1 gene (Takenaka *et al.* 2000).

Mechanism of regulation of IGFBP-1 gene expression by dietary proteins

Nuclear run-on assay of IGFBP-1 gene transcription elucidated that it is greatly increased in the liver of the rats fed on the protein-free diet, see Fig. 1 (Miura *et al.* 1993).

Extensive studies in many laboratories on the mechanism of the increase in the transcription rate of IGFBP-1 gene, employing gel mobility shift assay, DNase I protection
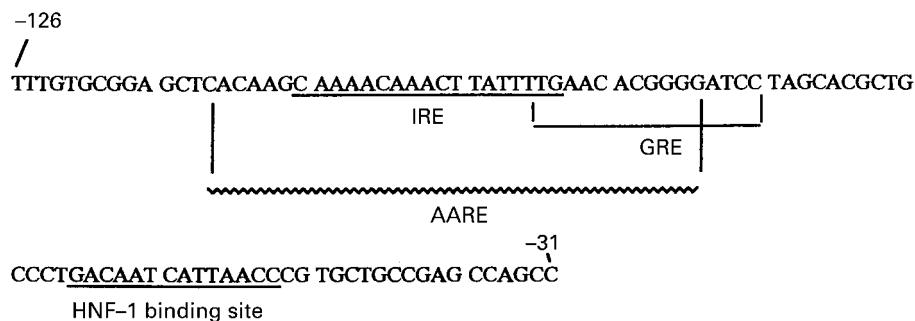

Fig. 2. A schematic representation of the IGFBP-1 gene promoter fragment from bp -126 to -31 from the transcription initiation site (Takenaka *et al.* 2000). Putative *cis*-regulatory elements; IRE (insulin responsive element), GRE (glucocorticoid responsive element), HNF-1 binding site and AARE (amino acid responsive element) are underlined.

assay and other techniques, have elucidated cAMP-responsive element, insulin-responsive element and gluco-corticoid responsive element in the 5′-upstream region of the IGFBP-1 gene (Fig. 2). Recently, we found a putative amino acid-responsive element (Fig. 2) (Takenaka *et al.* 2000). The results suggest that changes in the binding status of some transcription factor(s) to cAMP-responsive element, insulin-responsive element, glucocorticoid responsive element and amino acid-responsive element regulate the transcription of IGFBP-1 gene.

Conclusion

As described above, quantity and nutritional quality of dietary proteins extensively affect the expression of some genes. IGF-I, from our results and observations by other studies, is supposed to play an important role in the dietary regulation of body protein metabolism. Particularly, the expression of IGFBP-1 gene is regulated by dietary amino acids. The effect of dietary amino acids seems to be independent of insulin and glucocorticoids. IGFBP-1 gene has an amino acid response element(s) in it's regulatory region. Growth rate controlled by protein nutrition is now explained by the plasma concentration of IGF-I and IGFBP-1. The detailed mechanism of the changes in plasma IGF-I and IGFBP-1 concentrations is now explained at the molecular level. This means that the mechanism of the effect of protein nutrition on growth rate is being well understood biochemically and endocrinologically at the molecular level.

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